

promoter element SIE. Electroporation of a tyrosine-phosphorylated peptide, which interferes with both the association of **STAT** to the receptor and **STAT** dimerization, inhibits tubule formation in vitro without affecting either HGF-induced 'scattering' or growth. The same result is obtained using a specific '**decoy**' oligonucleotide that prevents **STAT** from binding to DNA and affecting the expression of genes involved in cell-cycle regulation (c-fos and waf-1). Activation of signal transducers that directly control transcription is therefore required for morphogenesis.

3/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09674336 98101492 PMID: 9440542

A thiol antioxidant regulates IgE isotype switching by inhibiting activation of nuclear factor-kappaB.

Yanagihara Y; Basaki Y; Kajiwar K; Ikizawa K

Clinical Research Center for Allergy, National Sagami Hospital, Sagami City, Kanagawa, Japan.

Journal of allergy and clinical immunology (UNITED STATES) Dec 1997, 100 (6 Pt 2) pS33-8, ISSN 0091-6749 Journal Code: 1275002

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The binding site for nuclear factor-kappaB (NF-kappaB) is present at the promoter region of the germline Cepsilon gene, but there is little information on whether this factor is involved in regulating IgE synthesis by human B cells. Accordingly, we studied the role of NF-kappaB in germline Cepsilon transcription by using two human Burkitt's lymphoma B cell lines, DND39 and DG75. In both cell lines, n-acetyl-L-cysteine (NAC), a potent thiol antioxidant, inhibited the triggering of the nuclear expression of NF-kappaB by IL-4 and by anti-CD40 monoclonal antibody. Although IL-4 activated signal transducers and activators of transcription (**STAT**) 6 in addition to NF-kappaB, NAC treatment or the transfection of **decoy oligodeoxynucleotides** for NF-kappaB or STAT6 only partly blocked IL-4-induced germline Cepsilon transcription. However, these two **decoy oligodeoxynucleotides** together almost completely abrogated IL-4-induced germline Cepsilon transcription. Of note, CD40-mediated enhancement of IL-4-driven germline Cepsilon transcription was markedly decreased by NAC or by a **decoy oligodeoxynucleotide** for NF-kappaB. The effect of NAC was also examined on deletional switch recombination underlying the isotype switch to IgE. NAC inhibited the generation of Smu/Sepsilon switch fragments in normal human B cells costimulated with IL-4 and anti-CD40 monoclonal antibody. It also abolished IL-4-induced upregulation of CD40 but promoted upregulation of CD23. These results suggest that coordination of NF-kappaB and STAT6 may be required for induction of germline Cepsilon transcription by IL-4, and that CD40-mediated NF-kappaB activation may be important in regulating both enhancement of germline Cepsilon transcription and class switching to IgE.

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File 155:MEDLINE(R) 1966-2003/Feb W1
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File 5:Biosis Previews(R) 1969-2003/Feb W1
(c) 2003 BIOSIS
*File 5: Alert feature enhanced for multiple files, duplicates
removal, customized scheduling. See HELP ALERT.

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>>>File 5 processing for OLIGO? stopped at OLIGONUCLEOTIDOHYDROLASE		
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	273914	OLIGO?
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3/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.

09674809 98101642 PMID: 9440692
Induction of epithelial tubules by growth factor HGF depends on the
STAT pathway.

Boccaccio C; Ando M; Tamagnone L; Bardelli A; Michieli P; Battistini C;
Comoglio P M

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Candiolo, Italy. cboccaccio@hal.ircc.polito.it

Nature (ENGLAND) Jan 15 1998, 391 (6664) p285-8, ISSN
0028-0836 Journal Code: 0410462

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Hepatocyte growth factor (HGF) induces a three-phase response leading to
the formation of branched tubular structures in epithelial cells. The HGF
receptor tyrosine kinase works through a Src homology (SH2) docking site
that can activate several signalling pathways. The first phase of the
response (scattering), which results from cytoskeletal reorganization, loss
of intercellular junctions and cell migration, is dependent on
phosphatidylinositol-3-OH kinase and Rac activation. The second phase
(growth) requires stimulation of the Ras-MAP kinase cascade. Here we show
that the third phase (tubulogenesis) is dependent on the **STAT**
pathway. HGF stimulates recruitment of **Stat** -3 to the receptor,
tyrosine phosphorylation, nuclear translocation and binding to the specific

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Processing

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S8	32	S7 AND VECTOR?
S9	20	RD (unique items)
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11/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09665977 98070321 PMID: 9405373

Cisplatin- and UV-damaged DNA lure the basal **transcription factor** TFIID/TBP.

Vichi P; Coin F; Renaud J P; Vermeulen W; Hoeijmakers J H; Moras D; Egly J M

Institut de Genetique et de Biologie Moleculaire et Cellulaire, BP 163, F-67404, Illkirch Cedex, Universite Louis Pasteur, Strasbourg, France.

EMBO journal (ENGLAND) Dec 15 1997, 16 (24) p7444-56, ISSN

0261-4189 Journal Code: 8208664

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

A connection between **transcription** and DNA repair was demonstrated previously through the characterization of TFIID. Using filter **binding** as well as in vitro **transcription** challenge **competition** assays, we now show that the promoter recognition **factor** TATA box-**binding** protein (TBP)/TFIID **binds** selectively to and is sequestered by cisplatin- or UV-damaged DNA, either alone or in the context of a larger protein complex including TFIID. Computer-assisted 3D structural analysis reveals a remarkable similarity between the structure of the TATA box as found in its TBP complex and that of either platinated or UV-damaged **oligonucleotides**. Thus, cisplatin-**treated** or UV-irradiated DNA could be used as a **competing binding site** which may lure TBP/TFIID away from its normal promoter sequence, partially explaining the phenomenon of DNA damage-induced inhibition of RNA synthesis. Consistent with an involvement of damaged DNA-specific **binding** of TBP in inhibiting **transcription**, we find that microinjection of additional TBP in living human fibroblasts alleviates the reduction in RNA synthesis after UV irradiation. Future anticancer drugs could be designed with the consideration of lesion recognition by TBP and their ability to reduce **transcription**.

11/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09654431 98085773 PMID: 9417870

Characterization of density-dependent regulation of the tyrosinase gene promoter: role of protein kinase C.

Mahalingam H; Watanabe A; Tachibana M; Niles R M

Department of Biochemistry and Molecular Biology, Marshall University School of Medicine, Huntington, West Virginia 25755, USA.

Experimental cell research (UNITED STATES) Nov 25 1997, 237 (1)

p83-92, ISSN 0014-4827 Journal Code: 0373226

Contract/Grant No.: CA-59539; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The rate-limiting step in melanogenesis is catalyzed by tyrosinase, a multifunctional enzyme encoded by the albino locus. We have previously reported that depletion of protein kinase C by long-term **treatment** of B16 mouse melanoma cells with phorbol dibutyrate (PDBu) prevented cell density-dependent melanogenesis. This was accompanied by a lack of induction of tyrosinase protein and mRNA. We report here the effect of PDBu on the functional activity of the mouse tyrosinase promoter by reporter gene assay and its effect on the **binding** of nuclear proteins from B16 cells to the "M-box" region of the mouse tyrosinase promoter. Short-term PDBu **treatment** of B16 cells transfected with a mouse tyrosinase promoter-luciferase construct resulted in increased reporter gene activity, while long-term PDBu **treatment** inhibited reporter gene activity. Using an **oligonucleotide** containing the M-box and its flanking residues in electrophoretic mobility shift assays, we found a density-dependent change in the pattern of DNA-protein complexes. One complex was found to be negatively regulated by long-term PDBu **treatment**. **Competition** experiments with various mutated **oligonucleotides** demonstrated that both the M-box and flanking residues are important for nuclear protein **binding**. The complex whose formation was inhibited by long-term PDBu **treatment** was shown to contain the basic helix-loop-helix leucine zipper protein microphthalmia-associated **transcription factor** (MITF). These results suggest that chronic PDBu **treatment** might inhibit tyrosinase expression (and subsequent melanogenesis) by affecting the amount or function of MITF.

11/3,AB/3 (Item 3 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09631961 98058978 PMID: 9395525

CCAAT/enhancer-**binding** protein delta activates insulin-like growth factor-I gene **transcription** in osteoblasts. Identification of a novel cyclic AMP signaling pathway in bone.

Umayahara Y; Ji C; Centrella M; Rotwein P; McCarthy T L McCarthy T L Yale U, New Haven, CT

Oregon Health Sciences University, Department of Medicine, Molecular Medicine Division, Portland, Oregon 97201-3098, USA.

Journal of biological chemistry (UNITED STATES) Dec 12 1997, 272

(50) p31793-800, ISSN 0021-9258 Journal Code: 2985121R

Contract/Grant No.: 5-PO1-HD20805; HD; NICHD; 5-RO1-DK37449; DK; NIDDK; 5-RO1-DK47421; DK; NIDDK

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Insulin-like growth factor-I (IGF-I) plays a key role in skeletal growth by stimulating bone cell replication and differentiation. We previously showed that prostaglandin E2 (PGE2) and other cAMP-activating agents enhanced IGF-I gene **transcription** in cultured primary rat

osteoblasts through promoter 1, the major IGF-I promoter, and identified a short segment of the promoter, termed HS3D, that was essential for hormonal regulation of IGF-I gene expression. We now demonstrate that CCAAT/enhancer-binding protein (C/EBP) delta is a major component of a PGE2-stimulated DNA-protein complex involving HS3D and find that C/EBPdelta transactivates IGF-I promoter 1 through this site. **Competition** gel shift studies first indicated that a core C/EBP half-site (GCAAT) was required for **binding** of a labeled HS3D oligomer to osteoblast nuclear proteins. Southwestern blotting and UV-cross-linking studies showed that the HS3D probe recognized a approximately 35-kDa nuclear protein, and antibody supershift assays indicated that C/EBPdelta comprised most of the PGE2-activated gel-shifted complex. C/EBPdelta was detected by Western immunoblotting in osteoblast nuclear extracts after **treatment** of cells with PGE2. An HS3D **oligonucleotide** **competed** effectively with a high affinity C/EBP site from the rat albumin gene for **binding** to osteoblast nuclear proteins. Co-transfection of osteoblast cell cultures with a C/EBPdelta expression plasmid enhanced basal and PGE2-activated IGF-I promoter 1-luciferase activity but did not stimulate a reporter gene lacking an HS3D site. By contrast, an expression plasmid for the related protein, C/EBPbeta, did not alter basal IGF-I gene activity but did increase the response to PGE2. In osteoblasts and in COS-7 cells, C/EBPdelta, but not C/EBPbeta, transactivated a reporter gene containing four tandem copies of HS3D fused to a minimal promoter; neither **transcription factor** stimulated a gene with four copies of an HS3D mutant that was unable to **bind** osteoblast nuclear proteins. These results identify C/EBPdelta as a hormonally activated inducer of IGF-I gene **transcription** in osteoblasts and show that the HS3D element within IGF-I promoter 1 is a high affinity **binding site** for this protein.

11/3,AB/4 (Item 4 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09328761 97232253 PMID: 9077528

Studies on the contribution of c-fos/AP-1 to arthritic joint destruction.
Shiozawa S; Shimizu K; Tanaka K; Hino K
Kobe University School of Medicine, Faculty of Health Science, Sumaku,
Japan.

Journal of clinical investigation (UNITED STATES) Mar 15 1997,
99 (6) p1210-6, ISSN 0021-9738 Journal Code: 7802877

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Features characteristic to rheumatoid joint destruction, including synovial overgrowth and bone resorption, are experimentally produced by augmenting c-fos gene expression. We tested here if arthritic joint destruction was inhibited upon inactivation of the c-fos/AP-1 signal by **administering** short double-stranded AP-1 DNA **oligonucleotides** into mice with collagen-induced arthritis to **compete** for the **binding** of AP-1 in vivo at the promoter **binding site**.

Arthritic joint destruction was inhibited in a sequence-specific and dose-dependent manner by **oligonucleotides** containing the AP-1 sequence. The **oligonucleotides** inhibited gene expression at the transcriptional level. Nucleotide sequences besides AP-1 also appeared to be important structurally ~~for binding of AP-1 onto DNA~~ and for the stability of **oligonucleotides** against nucleases. Immunohistochemical chase experiment **administering** biotinylated **oligonucleotides** into arthritic mice showed that AP-1 **oligonucleotides** reached the inflamed joint. Thus, activation of c-fos/AP-1 appears essentially important in arthritic joint destruction.

11/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09141546 97048020 PMID: 8892861

Identification of cis-acting sequences in the promoter of the herpes simplex virus type 1 latency-associated transcripts required for activation by nerve growth **factor** and sodium butyrate in PC12 cells.

Frazier D P; Cox D; Godshalk E M; Schaffer P A

Division of Molecular Genetics, Dana-Farber Cancer Institute, Boston, Massachusetts 02115, USA.

Journal of virology (UNITED STATES) Nov 1996, 70 (11) p7433-44

, ISSN 0022-538X Journal Code: 0113724

Contract/Grant No.: PO1AI24010; AI; NIAID; R37CA20260; CA; NCI; T32GM07196; GM; NIGMS; +

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

In the absence of detectable viral proteins, expression of the latency-associated transcripts (LATs) is likely regulated by cellular **factors** during latent infection of neurons with herpes simplex virus type 1. The amounts and activation states of these **factors** may in turn be regulated by extracellular regulatory **factors**. Consistent with this hypothesis, we have recently demonstrated that LAT expression is significantly enhanced by nerve growth **factor** (NGF) and sodium butyrate (NaB) in neurally derived PC12 cells. With the ultimate goal of identifying trans-acting cellular **factors** involved in regulating LAT expression during latency, we have attempted to identify the cis-acting elements to which these putative cellular **factors** bind by characterizing the LAT promoter and a series of 5' promoter deletion mutants in PC12 cells following **treatment** with the LAT-enhancing agents NGF and NaB. Transient expression assays demonstrated that distinct cis-acting sequences mediate basal and induced LAT promoter expression. Basal activity in PC12 cells is mediated by two elements: a negative regulatory element between -435 and -270 and a positive element between -240 and -204. The positive element contains **binding sites** for the transactivator Sp-1, whereas the negative element bears some resemblance to known neuron-specific silencer elements. In contrast to basal expression, maximum induction of the LAT promoter by NGF and NaB requires sequences between -159 and -81. Using gel mobility shift assays, we have identified three sets of protein-DNA complexes that **bind** to this 78-bp region and shown by **competition** analysis that **binding** is specific. The abundance and mobility of these complexes were altered by **treatment** with NGF or NaB. The nucleotide sequences to which these complexes **bind** were fine mapped by **competition** analysis with **oligonucleotide** probes containing substitution mutations. The target sequences identified exhibit no homology to **binding sites** of known **transcription factors**. These regions were critical for complex formation in vitro and for maximum induction of the LAT promoter by NGF and NaB in transient expression assays. The protein complexes that form with target sequences likely participate in the regulation of LAT expression in response to physiological stimuli in neurons in vivo.

11/3,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09102823 97021432 PMID: 8867792

Ultraviolet light and serum induce similar delayed responses that lead to activation of a mitogen-responsive promoter.

Hirsch S; Miskimins W K

Department of Biochemistry and Molecular Biology, University of South Dakota School of Medicine, Vermillion 57069, USA.

Cellular & molecular biology research (UNITED STATES) 1995, 41
(5) p441-9, ISSN 0968-8773 Journal Code: 9316986

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The transferrin receptor promoter is responsive to growth **factors** and mitogens. This induction is a delayed response and does not occur until several hours after stimulation of quiescent cells by mitogens. The results described here show that the transferrin receptor promoter is also activated by **treatment** with ultraviolet (UV) light. Activation of the promoter by UV light is dose dependent and requires the same cis-acting elements that are activated in response to serum and other mitogens. As with serum stimulation, activation of the promoter by UV light is a delayed event and is not initiated until 6 h after **treatment**. This coincides with the induction of nuclear **factors** that **bind** with specificity to the required cis-acting elements. A major GC-box **binding factor** induced by UV light is also induced by serum and has been shown to be supershifted by antibodies to the Sp1 **transcription factor**.

11/3,AB/7 (Item 7 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09030091 96400372 PMID: 8806748

Interactions of progesterone-dependent endometrial nuclear **factors** with the promoter of the rabbit uteroglobin gene.

Perez Martinez M; Garcia C; Lopez de Haro M S; Nieto A

Centro de Biologia Molecular Severo Ochoa, Facultad de Ciencias, Universidad Autonoma de Madrid, Spain.

Archives of biochemistry and biophysics (UNITED STATES) Sep 1 1996, 333 (1) p12-8, ISSN 0003-9861 Journal Code: 0372430

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

We have studied the in vitro interactions of a promoter fragment of the rabbit uteroglobin (UG) gene with endometrial nuclear **factors** from either control rabbits or progesterone-**treated** animals, a **treatment** that induces the **transcription** of the gene in the endometrium. Nuclear **factors** from liver, which does not express UG, were also compared. Using DNase I footprinting, several protected zones were observed on the promoter; some of these were common to all three nuclear extracts, whereas others seemed to be specific to progesterone **treated** endometrium. One of these footprints was over the TATA box region. A 28-bp synthetic **oligonucleotide** encompassing the sequence of that region was used as a probe in electrophoretic mobility shift assays (EMSA) and UV-crosslinking assays. In EMSA experiments, endometrial nuclear extracts, but not liver extracts, generated a major retarded complex that was strongly increased following progesterone stimulation. **Competition** experiments with unlabeled mutated versions of the probe showed that sequences 3' downstream from the TATA box (but not this motif) were responsible for the formation of the complex. UV-crosslinking experiments indicated that the probe interacted with two progesterone-dependent nuclear proteins of 55 and 60 kDa, different from the TATA box-**binding** protein. The results suggest that progesterone induction of the UG gene in the endometrium might be mediated by both general and tissue-specific nuclear **factors**.

11/3,AB/8 (Item 8 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08867212 96216375 PMID: 8621697

Activation of epidermal growth factor receptor gene transcription by phorbol 12-myristate 13-acetate is mediated by activator protein 2.

Johnson A C

Laboratory of Molecular Biology, Division of Basic Sciences, NCI, National Institutes of Health, Bethesda, Maryland 20892-4255, USA.

Journal of biological chemistry (UNITED STATES) Feb 9 1996, 271

(6) p3033-8, ISSN 0021-9258 Journal Code: 2985121R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The response of the epidermal growth factor (EGF) receptor gene to phorbol 12-myristate 13-acetate (PMA) was analyzed using nuclei and nuclear extracts prepared from PMA-treated KB cells. Transient transfection assays and nuclear run-off experiments showed that PMA increased EGF receptor gene transcription. Cell-free transcription with promoter mutants revealed that the region of the promoter containing nucleotides -150 to -16 was sufficient for PMA inducibility. A promoter fragment containing nucleotides -167 to -105 showed increased binding of a factor present in extracts prepared from PMA-treated cells. When this factor was partially purified by column chromatography, it showed specific PMA-dependent binding to an EGF receptor promoter fragment. This binding was competed by an SV40 fragment containing binding sites for Sp1, AP1, and AP2. Purified AP2 was used in DNase I footprinting experiments to show that this factor can bind to the EGF receptor promoter. Oligonucleotides corresponding to the AP2 binding sites found in the EGF receptor promoter showed the ability to bind AP2 and compete for the binding of a factor induced by PMA treatment. The addition of AP2 to nuclear extract resulted in increased transcription from the EGF receptor promoter. These results demonstrate that AP2 can activate EGF receptor gene expression and may mediate the PMA response of this gene.

11/3,AB/9 (Item 9 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

08843491 96200687 PMID: 8615671

Transcription factor decoy approach to decipher the role of NF-kappa B in oncogenesis.

Sharma H W; Perez J R; Higgins-Sochaski K; Hsiao R; Narayanan R
Division of Oncology, Roche Research Center, Hoffman-La Roche Inc., Nutley, NJ 07110, USA.

Anticancer research (GREECE) Jan-Feb 1996, 16 (1) p61-9,
ISSN 0250-7005 Journal Code: 8102988

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Antisense inhibition of the RelA subunit of NF-kappa B transcription factor (but not the NFkB1 subunit) causes pronounced inhibition of tumor cell growth in vitro and in vivo. Inhibition of either subunit, however, results in inhibition of the heterodimeric NF-kappa B complex in antisense-treated cells. Either of the subunits of NF-kappa B can form homo- or heterodimers with other members of the Rel oncogene family. In an effort to decipher the role of homo- vs heterodimeric NNF-kappa B in regulating tumor cell growth, we have used a decoy approach to trap these complexes in vivo. Using double-stranded phosphorothioates as a direct in vivo competitor for homo- vs heterodimeric NF-kappa B, we demonstrate that decoys more specific to RelA inhibit growth tumor cell growth in vitro. We demonstrate that RelA, either

as a homodimer or a heterodimer with some other members of the Rel family and not the classical NF-kappa B (RelA/NFkB1), is involved in the differential growth control of tumor cells. Our results indicate that such **transcription factor** decoys can be a non-antisense tool to study the function of DNA-binding transcription factors.

11/3,AB/10 (Item 10 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08841531 96198801 PMID: 8612540

Transforming growth factor -beta 1 responsiveness of the rat osteocalcin gene is mediated by an activator protein-1 binding site.

Banerjee C; Stein J L; Van Wijnen A J; Frenkel B; Lian J B; Stein G S
Department of Cell Biology, University of Massachusetts Medical Center,
Worcester 01655, USA.

Endocrinology (UNITED STATES) May 1996, 137 (5) p1991-2000,
ISSN 0013-7227 Journal Code: 0375040

Contract/Grant No.: AR-33920; AR; NIAMS; AR-39588; AR; NIAMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Osteocalcin (OC), a bone specific protein expressed during differentiation and mineralization of the bone extracellular matrix, is down-regulated upon treatment with transforming growth factor (TGF)-beta 1. To address the potential role of OC gene expression in relation to TGF-beta 1 regulation of bone formation and resorption, we examined the transcriptional activity of the rat OC promoter after TGF-beta 1 treatment. 5' deletion analysis of rat OC promoter-chloramphenicol acetyltransferase constructs demonstrated that TGF-beta 1 treatment repressed chloramphenicol acetyltransferase activity by 2.4-fold in transient transfections of ROS 17/2.8 cells. A 29-bp region between -162 and -134 identified as the TGF-beta 1 response domain, conferred TGF-beta 1 responsiveness to the -108 to +24 rat OC basal promoter in an orientation dependent manner. Mutation of an activator protein-1/cAMP-response element-like motif (-146 to -139) abolished TGF-beta 1 responsiveness of the construct. In vitro gel-mobility shift and competition assays using wild-type and mutated oligonucleotides and antibodies indicate that Fra-2, a Fos related transcription factor, binds to this motif. We show that Fra-2 is an activator of the OC promoter, and TGF-beta 1 inhibits this activation. Our results demonstrate that Fra-2 is hyperphosphorylated upon TGF-beta 1 treatment of ROS 17/2.8 cells. Additionally, treatment of cells with a staurosporine protein kinase C inhibitor abrogates TGF-beta 1 mediated down-regulation of the OC promoter activity. Together, these results demonstrate that TGF-beta 1 responsiveness of the rat osteocalcin gene in ROS 17/2.8 cells is mediated through an activator protein-1 like cis-acting element that interacts with Fra-2. Furthermore, our results are consistent with a critical role for TGF-beta 1 induced phosphorylation of Fra-2 in the repression of OC gene transcription.

11/3,AB/11 (Item 11 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08750483 96103579 PMID: 8524640

The transcription factors ATF-1 and CREB-1 bind constitutively to the hypoxia-inducible factor -1 (HIF-1) DNA recognition site.

Kvietikova I; Wenger R H; Marti H H; Gassmann M

Physiologisches Institut, Universitat Zurich-Irchel, Switzerland.

Nucleic acids research (ENGLAND) Nov 25 1995, 23 (22) p4542-50

, ISSN 0305-1048 Journal Code: 0411011

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The hypoxia-inducible **factor**-1 (HIF-1) was first described as a DNA **binding** activity that specifically recognizes an 8 bp motif known to be essential for hypoxia-inducible **erythropoietin** gene **transcription**. Subsequently HIF-1 activity has also been found in cell lines which do not express erythropoietin, suggesting that HIF-1 is part of a widespread oxygen sensing mechanism. In electrophoretic mobility shift assays HIF-1 DNA **binding** activity is only detectable in nuclear extracts of cells cultivated in a low oxygen atmosphere. In addition to HIF-1, a constitutive DNA **binding** activity also specifically **binds** the HIF1 probe. Here we report that CRE and AP1 **oligonucleotides** efficiently **competed** for **binding** of the HIF1 probe to this constitutive **factor**, whereas HIF-1 activity itself remained unaffected. Monoclonal antibodies raised against the CRE **binding factors** ATF-1 and CREB-1 supershifted the constitutive **factors** ATF-1 and CREB-1 supershifted the constitutive **factor**, while Jun and Fos family members, which constitute the AP-1 **factor**, were immunologically undetectable. Recombinant ATF-1 and CREB-1 proteins bound HIF1 probes either as homodimers or as heterodimers, indicating a new **binding** specificity for ATF-1/CREB-1. Finally, reporter gene assays in HeLa cells **treated** with either a cAMP analogue or a phorbol ester suggest that the PKA, but not the PKC signalling pathway is involved in oxygen sensing.

11/3,AB/12 (Item 12 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08731449 96070897 PMID: 7499234

Interplay of Sp1 and Egr-1 in the proximal platelet-derived growth **factor** A-chain promoter in cultured vascular endothelial cells.

Khachigian L M; Williams A J; Collins T

Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts 02115, USA.

Journal of biological chemistry (UNITED STATES) Nov 17 1995, 270

(46) p27679-86, ISSN 0021-9258 Journal Code: 2985121R

Contract/Grant No.: HL 35716; HL; NHLBI; HL 45462; HL; NHLBI; PO1 36028; PHS; +

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The platelet-derived growth **factor** (PDGF) A-chain has been implicated in the initiation and progression of vascular occlusive lesions. The elements in the human PDGF-A promoter that mediate increased expression of the gene in vascular endothelial cells have not been identified. A potent inducer of PDGF-A expression in endothelial cells is phorbol 12-myristate 13-acetate (PMA). 5'-Deletion and transfection analysis revealed that a G+C-rich region in the proximal PDGF-A promoter is required for PMA-inducible gene expression. This region bears overlapping consensus recognition sequences for Sp1 and Egr-1. PMA induces Egr-1 mRNA expression within 1 h, whereas PDGF-A transcript levels increase after 2-4 h. Constitutive levels of Sp1 are not altered over 24 h. A specific nucleoprotein complex is formed when an **oligonucleotide** bearing the G+C-rich element is incubated with nuclear extracts from PMA-**treated** cells. The temporal appearance of this complex is consistent with the transient increase in Egr-1 transcripts. Antibodies to Egr-1 completely supershift the PMA-induced complex. Interestingly, increased nuclear levels of Egr-1 attenuate the ability of Sp1 to interact with the **oligonucleotide**, implicating **competition** between Egr-1 and Sp1 for the G+C-rich element. Binding studies with recombinant proteins

demonstrate that Egr-1 can displace Sp1 from this region. Insertion of the G+C-rich element into a hybrid promoter-reporter construct confers PMA inducibility on the construct. Mutations that abolish Egr-1 **binding** also abrogate expression induced by PMA or overexpressed Egr-1. These findings demonstrate that PMA-induced Egr-1 displaces Sp1 from the G+C-rich element and activates expression driven by the PDGF-A proximal promoter in endothelial cells. The Sp1/Egr-1 displacement mechanism may be an important regulatory circuit in the control of inducible gene expression in vascular endothelial cells.

11/3,AB/13 (Item 13 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08716876 96079888 PMID: 7588292

Cis-regulatory elements conferring cyclic 3',5'-adenosine monophosphate responsiveness of the progesterone receptor gene in transfected rat granulosa cells.

Park-Sarge O K; Sarge K D

Department of Physiology, University of Kentucky, Lexington 40536-0084, USA.

Endocrinology (UNITED STATES) Dec 1995, 136 (12) p5430-7,
ISSN 0013-7227 Journal Code: 0375040

Contract/Grant No.: HD-30719; HD; NICHD

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

We have previously shown that both pituitary gonadotropins and forskolin induce progesterone receptor (PR) messenger RNA expression at the level of **transcription** in granulosa cells of the rat ovary. To determine the DNA regulatory elements that are important for **CAMP-induced transcription** of the PR gene in the ovary, we examined the **CAMP-induced** activity of promoter sequences in rat granulosa cells transfected with various fusion constructs containing PRB promoter sequences linked to the luciferase reporter gene. When cells were transfected with a luciferase fusion construct containing the 1375-base pair 5'-flanking region of the rat PRB gene, forskolin **treatment** substantially increased luciferase activity. Analysis of a series of 5'-deletion mutants indicated that a minimal PRB promoter containing 116 base pairs of upstream sequence (-116/3) was sufficient to increase luciferase activity in response to forskolin in transfected rat granulosa cells. This promoter contains a consensus CCAAT site in reverse orientation (5'-ATTGG-3') and a consensus GC box (5'-GGGGCGGGCC-3'), but no known **CAMP-responsive** element. Site-specific mutation of the GC box notably decreased both basal and **CAMP-induced** activity of this minimal PRB promoter. In addition, site-specific mutation of the CCAAT **binding site** within this proximal promoter of the PRB gene substantially decreased **CAMP-induced** activity, but did not significantly affect the basal activity of this promoter. Either mutation alone failed to abolish **CAMP** inducibility. In contrast, double mutation of both the GC box and the CCAAT box completely abolished **CAMP** inducibility, suggesting that the GC box and the CCAAT box act together to mediate **CAMP-induced transcription** of the PRB gene. Gel shift analysis shows that the minimal PRB promoter sequences form multiple complexes with nuclear proteins of granulosa cells, all of which are specifically **competed** by **oligonucleotides** containing the GC box and the CCAAT box. Taken together, our results suggest a functional role for **transcription factors** **binding** the GC box and the CCAAT box in mediating **CAMP-induced transcription** of the rat PRB promoter in rat granulosa cells.

11/3,AB/14 (Item 14 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08678438 96002857 PMID: 8536450

Biological activity in the repopulating rat spermatocyte after the withdrawal of **gossypol treatment**. VI. Alteration in nuclear factors for interaction with histone gene promoter.

Teng C S; Yang N Y; Chen Y

Department of Anatomy, North Carolina State University, Raleigh 27606, USA.

Contraception (UNITED STATES)

0010-7824 Journal Code: 0234361

Aug 1995, 52 (2) p129-34, ISSN

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

This article reports the effects of gossypol at the genomic level in rat spermatogenic cells. After gossypol **treatment** for various times (8, 12, and 19 weeks), the spermatogonial cells were allowed to rest for 2 to 4 weeks. The function of **histone H4 gene promoter (H4GP)** in the repopulating pachytene spermatocytes (RPS) was investigated. The sequences of the **oligonucleotides** for the H4GP **binding sites** 1 and 2 were synthesized by an ABI-392 DNA synthesizer. RPS and the control pachytene spermatocytes (CPS) were obtained by centrifugal elutriation and subsequently they were used for the preparation of nuclear protein extracts (NPE). The NPE interaction with the DNA fragment of site 1 or 2 was studied by an electrophoresis mobility shift assay (EMSA). EMSA with NPE-CPS revealed ten major gel shift bands for site 1 and 2. The presence of extra unlabelled DNA fragments **competed** with 6 of the bands. After 2 to 4 weeks recovery from 8, 12, and 19 weeks of gossypol **treatment**, NPE-RPS failed to shift four bands (b through e) in site 1. These results suggested that gossypol **treatment** affected the **transcription factors** for interaction with site 1. On the contrary, no effect was demonstrated in NPE that interacted with site 2. Furthermore, gossypol **treatment** did not change the nucleotide sequence in the H4GP site 1 and 2.

11/3,AB/15 (Item 15 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08576752 95336453 PMID: 7541987

The Ah receptor recognizes DNA **binding sites** for the B cell **transcription factor**, BSAP: a possible mechanism for dioxin-mediated alteration of CD19 gene expression in human B lymphocytes.

Masten S A; Shiverick K T

Department of Pharmacology and Therapeutics, University of Florida, Gainesville 32610-0267, USA.

Biochemical and biophysical research communications (UNITED STATES) Jul 6 1995, 212 (1) p27-34, ISSN 0006-291X Journal Code: 0372516

Contract/Grant No.: HD-18506; HD; NICHD

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) inhibits murine and human B lymphocyte immunoglobulin production through an unknown mechanism. This study investigated the effect of TCDD on expression of the CD19 gene in a human B lymphocyte cell line. Northern blot analysis showed that TCDD **treatment** decreased steady state levels of CD19 mRNA by 67% in the IM-9 cell line. Using a gel mobility shift assay, we identified a DNA-**binding** complex in IM-9 nuclear extracts that by several criteria appears to be the Ah receptor. In addition, the Ah receptor complex recognized a DNA **binding site** for B cell lineage-specific activator protein (BSAP) in the promoter region of the human CD19 gene which is similar to the consensus Ah receptor DNA **binding site**.

These results suggest that the AhR could interfere with BSAP-stimulated CD19 gene **transcription** by **competition** for a common DNA **binding site**.

11/3,AB/16 (Item 16 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08356741 95118299 PMID: 7818486

Activation of Ito cells involves regulation of AP-1 **binding** proteins and induction of type I collagen gene expression.

Armendariz-Borunda J; Simkevich C P; Roy N; Raghow R; Kang A H; Seyer J M
VA Medical Center Research Service, Memphis, TN 28104.

Biochemical journal (ENGLAND) Dec 15 1994, 304 (Pt 3) p817-24,

ISSN 0264-6021 Journal Code: 2984726R

Contract/Grant No.: AR-39166-06; AR; NIAMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Activation of liver Ito cells is characterized by increased proliferation, fibrogenesis, loss of cellular retinoid and change of cell-shape. Here, we have described fundamental differences between freshly isolated Ito cells (FIC) and long-term cultured Ito cells (LTIC). This process of activation correlates with the absence of expression of Pro alpha 1(I) gene in FIC. LTIC expressed abundant transcripts of Pro alpha 1(I) gene. Nuclear run-off experiments showed the inability of FIC to support Pro alpha 1(I) RNA **transcription** while LTIC transcribed it greater than 5-fold as compared with FIC. Transforming growth factor beta (TGF beta)-**treated** LTIC had a preferential increase in the rate of Pro alpha 1(I) gene **transcription** as compared with control LTIC. A human collagen type I promoter-enhancer construct (pCOL-KT) [Thompson, Simkevich, Holness, Kang and Raghow (1991) J. Biol. Chem. 266, 2549-2556] was readily expressed in LTIC but failed to be expressed in FIC. Furthermore, TGF beta **treatment** of LTIC resulted in an increased expression of pCOL-KT. The deletion of an activator protein-1 (AP-1) **binding site** (+598 to +604) in the 360 bp enhancer region of pCOL-KT (S360) caused decreased expression of the CAT reporter gene, suggesting that this bonafide AP-1 site can, at least in part, mediate the transactivation effect of TGF beta. Using DNAase I protection, we demonstrate a single foot-print located at +590 to +625 in the S360 fragment; nuclear extracts prepared from TGF beta-**treated** LTIC exhibited greater activity of these AP-1 **binding** proteins. Gel mobility assays corroborated and extended the footprinting observation. No AP-1-**binding** activity was found in the nuclear extracts of FIC. Double-stranded **oligonucleotides** containing the consensus AP-1 motif were able to **compete** out the **binding**; consensus NF-1 motif **oligonucleotides** failed to do so. The preincubation of nuclear extracts from control and TGF beta-**treated** LTIC with antibodies against c-jun and c-fos rendered a reduced **binding** of AP-1 proteins to the target S360 fragment.

11/3,AB/17 (Item 17 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08351072 95110340 PMID: 7811280

Cardiac myocyte differentiation induced by 3,5,3'-triiodo-L-thyronine (T3) in P19 teratocarcinoma cells is accompanied by preferential **binding** of RGG(T/A)CA direct repeats spaced by 4 base pairs in the DNA.

Rodriguez E R; Tan C D; Onwuta U S; Parrillo J E

Section of Cardiology, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL.

Biochemical and biophysical research communications (UNITED STATES) Dec
30 1994, 205 (3) p1899-906, ISSN 0006-291X Journal Code:
0372516

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

When **treated** with T3, P19 cells differentiate into ultrastructurally proven cardiac myocytes and express the cardiac ventricular specific marker ventricular myosin light chain 2V. This differentiation is irreversibly induced in culture during the first 48 hrs of exposure to T3. We studied the **binding** of P19-indigenous **transcription factors** of the Steroid-Thyroid-Retinoic superfamily of nuclear receptors to **oligonucleotide** response elements bearing direct, inverted and palindromic repeats of the consensus sequence RGG(T/A)CA. Electrophoretic mobility shift assays showed a preference in T3-treated P19 cells for **binding** RGG(T/A)CA "half sites" in direct repeat orientation separated by 4 base pairs. The specificity of **binding** was confirmed in **competition** experiments. This finding suggests that target genes bearing thyroid response elements spaced by 4 base pairs in their promoter regions play an important role in the cardiac differentiation induced by T3 in P19 teratocarcinoma cells.

11/3,AB/18 (Item 18 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08184799 94321434 PMID: 8045940

Identification of a DNA sequence involved in osteoblast-specific gene expression via interaction with helix-loop-helix (HLH)-type **transcription factors**.

Tamura M; Noda M

Department of Molecular Pharmacology, Tokyo Medical and Dental University, Japan.

Journal of cell biology (UNITED STATES) Aug 1994, 126 (3)
p773-82, ISSN 0021-9525 Journal Code: 0375356

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

To elucidate regulatory mechanism(s) underlying differentiation of osteoblasts, we examined involvement of helix-loop-helix (HLH)-type **transcription factors** in osteoblast-specific expression of a phenotypic marker gene which encodes osteocalcin, a major noncollagenous bone matrix protein, exclusively expressed in osteoblasts. Overexpression of a dominant negative HLH protein, Id-1, decreased the activity of the 1.1-kb osteocalcin gene promoter cotransfected into rat osteoblastic osteosarcoma ROS17/2.8 cells. Analysis of deletion mutants revealed that a 264-bp fragment of osteocalcin promoter (-198 to +66) was sufficient for the Id-1-dependent suppression. Furthermore, the activity of the same promoter fragment (-198 to +66) was enhanced when antisense Id-1 expression vector was cotransfected. This osteocalcin gene promoter region contains two sites of an E-box motif, a consensus **binding site** for HLH proteins, which we refer to as OCE1 (CACATG, at -102) and OCE2 (CAGCTG, at -149), respectively. Mutagenesis in OCE1 but not OCE2 led to greater than 50% reduction in transcriptional activity of the osteocalcin gene promoter. Electrophoresis mobility shift assay indicated that **factors** in nuclear extracts prepared from ROS17/2.8 cells bound to the 30-bp **oligonucleotide** probe containing the E-box motif of OCE1. This **binding** was **competed** out by OCE1 **oligonucleotide** but neither by OCmE1 **oligonucleotide** in which E-box motif was mutated nor by OCE2. The OCE1-**binding** activity in the nuclear extracts of ROS17/2.8 cells was reduced by 70% when bacterially expressed Id-1 protein was added to the reaction mixture, suggesting the involvement of HLH

proteins in the DNA/protein complex formation. In contrast to the osteoblast-like cells, OCE1-binding activity in the nuclear extracts of C3H10T1/2 fibroblasts was very low. However, when these fibroblasts were **treated** with recombinant human bone morphogenetic protein-2 which induced expression of osteocalcin as well as other phenotypic markers of osteoblasts, OCE1-binding activity was increased approximately 40-fold, indicating that OCE1 would be involved in the tissue-specific expression of the osteocalcin gene. These findings indicated for the first time that osteoblast-specific gene **transcription** is regulated via the interaction between certain E-box **binding transcription factor(s)** in osteoblasts and the OCE1 sequence in the promoter region of the osteocalcin gene.

11/3,AB/19 (Item 19 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08166574 94300221 PMID: 8027667

Interaction of nuclear proteins with an AP-1/CRE-like promoter sequence in the human TNF-alpha gene.

Newell C L; Deisseroth A B; Lopez-Berestein G

Department of Clinical Investigation, University of Texas M. D. Anderson Cancer Center, Houston, TX 77030.

Journal of leukocyte biology (UNITED STATES) Jul 1994, 56 (1)

p27-35, ISSN 0741-5400 Journal Code: 8405628

Contract/Grant No.: CA09598-5; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The tumor necrosis **factor** alpha (TNF-alpha) promoter contains an AP-1/CRE-like **binding site**, TGAGCTCA. AP-1 elements generally transduce signals involving protein kinase C; the CRE site mediates a cAMP response, involving protein kinase A. Thus, this element has the potential to receive signals through divergent signaling pathways. Nuclear protein **binding** studies using extracts from THP-1 monocytic cells **treated** with lipopolysaccharide (LPS), which stimulates, or dexamethasone (Dex) or pentoxifylline (PTX), which inhibit TNF production, respectively, suggest that two low-mobility complexes could be involved in regulation through this promoter region. PTX and Dex increase **binding** of both these complexes compared with untreated cells; approximately 2 hours after LPS induction, the upper complex becomes undetectable. This upper complex is composed of ATF2 (activating **transcription factor** 2, a cyclic AMP responsive element **binding** protein) homodimers; the lower is a heterodimer of jun/ATF2. LPS induces c-jun and thus may enhance formation of jun/ATF2 complexes, which could be activating complexes. In this case, the simultaneous presence of both complexes, which would occur in the presence of Dex or PTX, could reduce the amount of TNF **transcription** through **competitive binding**. Through in vitro **competitive binding** studies comparing the **binding** affinities of the TNF promoter sequence and a consensus CRE, we further suggest how variation of endogenous **binding** sequences from consensus may be an important property for regulatory control of particular genes.

11/3,AB/20 (Item 20 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08120517 94253188 PMID: 8195246

Estrogen receptor-Sp1 complexes mediate estrogen-induced cathepsin D gene expression in MCF-7 human breast cancer cells.

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Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station 77843-4466.

Journal of biological chemistry (UNITED STATES) Jun 3 1994, 269
(22) p15912-7, ISSN 0021-9258 Journal Code: 2985121R

Contract/Grant No.: ES04176; ES; NIEHS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Cathepsin D is an estrogen (17 beta-estradiol, E2)-inducible lysosomal protease. A putative estrogen receptor (ER)-Sp1-like sequence (GGGCGG(n)23ACGGG) has been identified in the non-coding strand of the cathepsin D promoter (-199 to -165), and electromobility shift assays of nuclear extracts from MCF-7 and HeLa cells confirm that both the ER and Sp1 protein **bind** to 32P-labeled ER/Sp1 oligo. For example, nuclear extracts from MCF-7 cells **bind** to the 32P-labeled ER/Sp1 oligo; however, ER/Sp1 **binding** can be decreased by selective **competition** with excess unlabeled estrogen responsive element and Sp1 oligos, immunodepletion with ER or Sp1 antibodies, and by **treating** cells with ICI 164,384, an antiestrogen which inhibits formation of ER homodimer. Moreover, E2-induced chloramphenicol acetyltransferase (CAT) activity in MCF-7 cells cotransfected with a human estrogen receptor expression plasmid and a plasmid containing an ER/Sp1 sequence cloned upstream to a thymidine kinase promoter and a CAT reporter. In cotreatment studies, ICI 164,384 inhibited E2-induced CAT activity. In contrast, E2 did not induce CAT activity in MCF-7 cells transfected with plasmids containing mutations in the ER or Sp1 segments of the ER/Sp1 oligo, thus confirming that both cognate **binding sites** are required for estrogen responsiveness.

11/3,AB/21 (Item 21 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08046899 94192856 PMID: 8143900

Characterization of a corticotropin releasing hormone responsive region in the murine proopiomelanocortin gene.

Bishop J F; Mouradian M M

Experimental Therapeutics Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892.

Molecular and cellular endocrinology (IRELAND) Nov 1993, 97

(1-2) p165-71, ISSN 0303-7207 Journal Code: 7500844

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The most potent, physiologic activator of proopiomelanocortin (POMC) gene **transcription** is corticotropin releasing hormone (CRH) and increased intracellular cAMP is critical for this effect. The 5'-flanking region of the murine POMC gene has several potential **binding sites** for regulatory proteins. To characterize the region between nucleotides -141 and -106, which includes a TRE-like site and an adjacent AP-2 consensus sequence, and to study its role in signal-**transcription** coupling, gel mobility shift assays and transient expression of CAT chimeras were performed. In transient transfections of AtT-20 cells with pCATp-141/-106, CRH **treatment** led to significant increases in CAT expression compared with CRH **treatment** of cells transfected with the enhancerless vector. However, no response to direct activation of cAMP dependent protein kinase or protein kinase C was detected. Despite the high homology of the sequence -137/-131 to the consensus AP-1 **binding site** (TRE), the nuclear **factor(s)** in AtT-20 cells **binding** to this region appears to be different than authentic AP-1 since neither a **competitor oligonucleotide** having the authentic TRE sequence nor antibodies against Jun or Fos affected the gel shift pattern of a probe having the -137/-131 sequence. We conclude that the -141 to -106 region of the murine POMC gene contains a functional CRH responsive element and that second

messenger systems that transduce the CRH signal to this element do not exert their actions solely through activation of PKA or PKC.

11/3,AB/22 (Item 22 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08009413 94146013 PMID: 8312279

Regulatory mechanism of human **factor IX** gene: protein **binding**
at the Leyden-specific region.

Kurachi S; Furukawa M; Salier J P; Wu C T; Wilson E J; French F S;
Kurachi K

Department of Human Genetics, University of Michigan Medical Center, Ann Arbor 48109-0618.

Biochemistry (UNITED STATES) Feb 15 1994, 33 (6) p1580-91,
ISSN 0006-2960 Journal Code: 0370623

Contract/Grant No.: HL 38644; HL; NHLBI

Erratum in Biochemistry 1995 Oct 31;34(43) 14270

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Hemophilia B-Leyden is characterized by the gradual amelioration of bleeding after the onset of puberty. All Leyden phenotype mutations found to date lie within the Leyden-specific region, which spans roughly nt-40 to +20 in the 5' end of the human **factor IX** gene. With HepG2 cell nuclear extracts, the Leyden-specific region and its immediate neighboring region of the normal **factor IX** gene showed five DNase I footprints: FP-I (nt +4 to +19), FP-II (nt -16 to -3), FP-III (nt -27 to -19), FP-IV (nt -67 to -49), and FP-V (nt -99 to -77). Protein **binding** affinities of short **oligonucleotides** containing sequences of FP-I, FP-II, or FP-III were substantially reduced in the presence of Leyden phenotype mutations in these areas, correlating well with the negative effects of these mutations on **factor IX** gene expression. A Leyden phenotype mutation at nt -20 (T to A) caused a loss of both footprints FP-III and FP-II but generated a new footprint, FP-III' (nt -34 to -23), partially overlapping with FP-III, indicating mutation-dependent **competitive protein binding** at these sites. Although the FP-III' area contains an androgen responsive element-like sequence, the nuclear protein that **binds** at FP-III' is not androgen receptor. The protein was not recognized by anti-androgen receptor antibody and, furthermore, was present not only in liver but also in both androgen receptor-positive and androgen receptor-negative cells in electrophoretic mobility shift assays. The nuclear concentration of this protein increased significantly upon **treatment** of the HepG2 cells with testosterone. Its **binding** affinity to an **oligonucleotide** (-32sub) containing the FP-III' sequence was greatly reduced in the presence of exogenous androgen receptor, suggesting a possible interaction of this protein with androgen receptor. The affinities of both this protein and a protein which **binds** to FP-III (presumably HNF-4) to -32sub with a mutation at nt -26 were grossly lowered. These findings suggest that the amelioration of hemophilia B-Leyden with a mutation at nt -20 after puberty involves **binding** of a specific non-androgen receptor nuclear protein at FP-III' and it is able to substitute for the function of a protein bound at FP-III in the normal gene optimally through its elevated interaction with androgen receptor upon a surge of testosterone. (ABSTRACT TRUNCATED AT 400 WORDS)

11/3,AB/23 (Item 23 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07788579 93314686 PMID: 8325323

An NF-kappa B-like element plays an essential role in

interleukin-1-mediated costimulation of the mouse interleukin-2 promoter.
Stricker K; Serfling E; Krammer P H; Falk W
Institute for Immunology and Genetics, German Cancer Research Center,
Heidelberg, FRG.

European journal of immunology (GERMANY) Jul 1993, 23 (7)
p1475-80, ISSN 0014-2980 Journal Code: 1273201

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Interleukin-1 (IL-1) costimulation is required for efficient IL-2 synthesis and IL-2 receptor (IL-2R) expression of T cells. The molecular events leading to these effects are largely unknown. We utilized an IL-1-responsive and an IL-1-non-responsive subclone of the mouse thymoma cell line EL4 to investigate how IL-1 activates IL-2 gene expression. We correlated IL-2 promoter activity with the activity of the endogenous IL-2 gene, thereby showing the biological significance of our results. Our experiments provide new functional data showing that a major target of IL-1 mediated costimulation is the chi B-like site, T cell element distal TCED (GGGATTTTCAC), of the IL-2 promoter. Thus, deletion or mutation of TCED within a complete IL-2 promoter abrogated IL-1 costimulation in the IL-1 responsive EL4 subclone. Therefore, the TCED element is functionally essential for the effect of IL-1. We also identified a nuclear **factor** (NF), IL-1 NF, that **binds** to the TCED site after IL-1 stimulation.

This **factor** was only present in the IL-1-responsive EL4 subclone and not in the IL-1-non-responsive subclone after IL-1 stimulation and did not appear after phytohemagglutinin (PHA)-**treatment**. **Binding** of IL-1 NF to the TCED site was **competed** by a typical chi B **oligonucleotide**, suggesting that it is similar to NF-chi B in its **DNA-binding** properties. However, the TCED element was only activated by costimulation with PHA and IL-1 whereas a typical chi B element was already activated by IL-1 alone. These data suggest that the biological function of the TCED element of the IL-2 promoter differs from that of a canonical chi B element. Our data provide new evidence that IL-1 acts on the IL-2 promoter by activating the TCED element via the **transcription factor** IL-1 NF. Furthermore, activation of this element requires two signals, delivered by IL-1 and PHA, in this way reflecting the activation requirement for the endogenous IL-2 gene.

11/3,AB/24 (Item 24 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07195614 92119240 PMID: 1531033

The nuclear **factor** SP8BF **binds** to the 5'-upstream regions of three different genes coding for major proteins of sweet potato tuberous roots.

Ishiguro S; Nakamura K
Laboratory of Biochemistry, School of Agriculture, Nagoya University,
Japan.

Plant molecular biology (NETHERLANDS) Jan 1992, 18 (1) p97-108
, ISSN 0167-4412 Journal Code: 9106343

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Sporamin and beta-amylase are two major proteins of tuberous roots of sweet potato, and expression of genes coding for sporamin and beta-amylase is induced concomitantly in leaves with the petioles attached by exogenous supply of sucrose or polygalacturonic acid. We have used a DNase I footprinting assay to characterize nuclear **factors** that **bind** to the 5'-upstream regions of gSPO-A1, gSPO-B1 and g beta-Amy genes that encode A-type sporamin, B-type sporamin and the subunit of beta-amylase, respectively. Nuclear extracts from sucrose-**treated** petioles

protected a region around -155 relative to the **transcription** start site of gSPO-A1 and a region around -880 of g beta-Amy from DNase I digestion on both strands. These two protected regions both contained the sequence ACTGTGTA, designated SP8a, in opposite orientation with respect to the direction of **transcription**. A gel mobility shift assay with SP8a **oligonucleotide** and **competition** experiments indicated that a common **factor** SP8BF binds to the SP8a sequence in gSPO-A1 and g beta-Amy. **Binding** of SP8BF to the SP8a **oligonucleotide** was abolished by mutation within the SP8a sequence. Fragments of the 5'-upstream region of gSPO-B1 also **competed** for the **binding** of SP8BF to the SP8a **oligonucleotide**, and the DNase I footprinting assay revealed three **binding sites** for SP8BF in the 5'-upstream region of gSPO-B1. These three sites in gSPO-B1 all contained the sequence TACTATT, designated SP8b, which shared 4 nucleotides at identical positions with the SP8a sequence. An inverted repeat of the SP8b sequence was also present at one protected site in the 5'-upstream region of g beta-Amy. In addition to sucrose-treated petioles, SP8BF activity was also present in tuberous roots and untreated fresh petioles of sweet potato. Furthermore, the activity was also detected in stems of tobacco plantlets, suggesting that SP8BF is an ubiquitous **factor**.

11/3,AB/25 (Item 25 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07086317 92017873 PMID: 1922089

Androgen modulation of DNA-binding factors in the mouse kidney.

Rhee M; Dimaculangan D; Berger F G
Department of Biological Sciences, University of South Carolina, Columbia 29208.

Molecular endocrinology (Baltimore, Md.) (UNITED STATES) Apr 1991
5 (4) p564-72, ISSN 0888-8809 Journal Code: 8801431
Contract/Grant No.: DK-37265; DK; NIDDK
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Transcription of the RP2 gene in the mouse kidney is induced by androgens. This induction is species specific within the genus Mus. For example, the gene responds to androgens in Mus domesticus, but is refractory to hormone in the distantly related species M. caroli. In the present report we have characterized DNA-binding factors that recognize the 5' flanking region of the RP2 gene. One **factor** (termed RPBPF-1) binds a DNA fragment spanning the region between -157 and -311 relative to the transcriptional start site. RPBPF-1 is present in kidney nuclear extracts from both control and androgen-treated M. domesticus as well as from control M. caroli; however, in the latter species a distinct **factor** (termed RPBPF-2) is induced by androgens and replaces RPBPF-1. The androgen-dependent replacement of RPBPF-1 by RPBPF-2 is specific to the kidney of M. caroli. DNase-1 footprinting analyses indicate that the two **factors** recognize distinct, yet overlapping, regions of the RP2 promoter: RPBPF-1 binds the region between -247 and -269, while RPBPF-2 binds the region between -265 and -290. The RPBPF-2-binding site contains a sequence that is homologous to that recognized by nuclear **factor**-1 (NF-1), suggesting that RPBPF-2 is a NF-1-like **factor**. This is supported by **competition** experiments with synthetic **oligonucleotides** corresponding to the NF-1-binding site within the adenovirus origin of replication. Thus, androgens can modulate, in a species- and tissue-specific manner, DNA-binding factors that recognize promoter regions of genes. (ABSTRACT TRUNCATED AT 250 WORDS)

11/3,AB/26 (Item 26 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

06805564 91112793 PMID: 1846513

Mechanism of action of 2,3,7,8-tetrachlorodibenzo-p-dioxin antagonists: characterization of 6-[125I]methyl-8-iodo-1,3-dichlorodibenzofuran-Ah receptor complexes.

Piskorska-Pliszczynska J; Astroff B; Zacharewski T; Harris M; Rosengren R; Morrison V; Safe L; Safe S

Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station 77843-4466.

Archives of biochemistry and biophysics (UNITED STATES) Jan 1991, 284 (1) p193-200, ISSN 0003-9861 Journal Code: 0372430

Contract/Grant No.: ES03554; ES; NIEHS; ES03843; ES; NIEHS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

6-Methyl-8-iodo-1,3,-dichlorodibenzofuran (I-MCDF) and its radiolabeled analog [125I]MCDF have been synthesized and used to investigate the mechanism of action of 1,3,6,8-substituted dibenzofurans as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) antagonists. Like 6-methyl-1,3,8-trichlorodibenzofuran (MCDF), I-MCDF partially antagonized the induction by TCDD of microsomal aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin O-deethylase (EROD) activities in rat hepatoma H-4-II E cells and male Long-Evans rat liver. Incubation of rat liver cytosol with [125I]MCDF followed by velocity sedimentation analysis on sucrose gradients gave a specifically bound peak which sedimented at 9.6 S. This radioactive peak was displaced by incubation with a 200-fold excess of unlabeled I-MCDF, 6-methyl-1,3,8-trichlorodibenzofuran (MCDF), 2,3,7,8-tetrachlorodibenzofuran (TCDF), and benzo [a]pyrene. Based on the velocity sedimentation results and the elution profile from a Sephacryl S-300 gel permeation column, the Stokes radius and apparent molecular weights of the cytosolic [125I]MCDF-Ah receptor complex were 6.5 nm and 259,200, respectively. In addition, the nuclear [125I]MCDF-receptor complex eluted at a salt concentration of 0.29 M KCl from a DNA-Sepharose column. Velocity sediment analysis of the nuclear [125I]MCDF-Ah receptor complex from rat hepatoma H-4-II E cells gave a specifically bound peak at 5.6 +/- 0.8 S. All of these properties were similar to those observed using [3H]TCDD as the radioligand. In addition, there were several ligand-dependent differences observed in the properties of the I-MCDF and TCDD receptor complexes; for example, the [125I]MCDF rat cytosolic receptor complex was unstable in high salt buffer and was poorly transformed into a form with increased binding affinity on DNA-Sepharose columns; Scatchard plot analysis of the saturation binding of [3H]TCDD and [125I]MCDF with rat hepatic cytosol gave KD values of 1.07 and 0.13 nM and Bmax values of 137 and 2.05 fmol/mg protein, respectively. The nuclear extract from rat hepatoma H-4-II E cells treated with I-MCDF or TCDD interacted with a dioxin-responsive element in a gel retardation assay. These results suggest that the mechanism of antagonism may be associated with competition of the antagonist receptor complex for nuclear binding sites.

11/3,AB/27 (Item 27 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

06772810 91088269 PMID: 2175884

BAP, a rat liver protein that activates transcription through a promoter element with similarity to the USF/MLTF binding site.

Kugler W; Kaling M; Ross K; Wagner U; Ryffel G U

Kernforschungszentrum Karlsruhe, Institut fur Genetik und Toxikologie, Karlsruhe, FRG.

Nucleic acids research (ENGLAND) Dec 11 1990, 18 (23) p6943-51, ISSN 0305-1048 Journal Code: 0411011

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The vitellogenin genes of *Xenopus* are liver-specifically expressed. An in vitro **transcription** system derived from rat liver nuclei allowed us to define the cis-element BABS (B-activator **binding site**) in the promoter of the B1 vitellogenin gene. An **oligonucleotide** encompassing the region from -53 to -44 linked to a TATA box is sufficient for a tenfold increase of the transcriptional activity. Gel retardation assays with nuclear rat liver proteins reveal two DNA-protein complexes: Complex 1 can be **competed** by the USF/MLTF **binding site** of the adeno major late promoter whereas complex 2 is a distinct protein we refer to as BAP (B-activator protein). In vitro **transcription** experiments in the presence of USF/MLTF **binding site** as **competitor** show that BAP is an efficient **transcription factor**. Based on UV cross-linking we estimate that BAP has a molecular weight of 58 kd. Phosphatase **treatment** reveals that DNA **binding** of BAP requires phosphorylation. BABS is also present in the hepatitis B virus enhancer suggesting that it might play a role in the tumorigenic potential of the virus.

11/3,AB/28 (Item 28 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

06751778 91061734 PMID: 2174104

Interleukin-1-mediated enhancement of mouse **factor B** gene expression via NF kappa B-like hepatoma nuclear **factor**.

Nonaka M; Huang Z M

Department of Immunobiology, Kanazawa University, Japan.

Molecular and cellular biology (UNITED STATES) Dec 1990, 10

(12) p6283-9, ISSN 0270-7306 Journal Code: 8109087

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Complement **factor B**, a serine protease playing a pivotal role in alternative pathway activation, is an acute-phase plasma protein. Previous studies have revealed that interleukin-1 (IL-1) mediates, at least in part, the acute-phase induction of **factor B** expression and that the IL-1-responsive element resides in the region between -553 and -478 relative to the **transcription** initiation site of the mouse **factor B** gene. In this paper, we demonstrate a specific **binding site** for a nuclear **factor** of human hepatoma HepG2 cells in this region of the **factor B** gene, using gel shift and methylation interference analysis. The nucleotide sequence of the **binding site** is closely similar to the NF kappa B or H2TF1 **binding motif**. The **binding** activity of HepG2 showed very similar specificity to that of NF kappa B or H2TF1, as shown by a **competition binding** assay, and was induced by IL-1 alpha **treatment**. A synthetic **oligonucleotide** corresponding to this **binding site**, as well as a similar sequence found in another class III complement C4 gene, conferred IL-1 responsiveness on the minimal **factor B** promoter. In contrast, a mutated **oligonucleotide** that could not **bind** to the HepG2 nuclear **factor** did not confer IL-1 responsiveness. These results suggest that IL-1 induces **factor B** expression via NF kappa B or a closely related **factor** in hepatocyte nuclei.

11/3,AB/29 (Item 29 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

06591083 90291988 PMID: 2162764

Nuclear **factor I** acts as a **transcription factor** on the MMTV promoter but **competes** with steroid hormone receptors for DNA **binding**.

Bruggemeier U; Rogge L; Winnacker E L; Beato M

Institut für Molekularbiologie und Tumorforschung, Philipps Universität, Marburg, FRG.

EMBO journal (ENGLAND) Jul 1990, 9 (7) p2233-9, ISSN 0261-4189 Journal Code: 8208664

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Several steroid hormones induce **transcription** of the mouse mammary tumor virus (MMTV) promoter, through an interaction of their respective receptors with the hormone responsive elements (HREs) in the long terminal repeat (LTR) region. The molecular mechanism underlying transcriptional activation is not known, but **binding** of nuclear **factor I** (NFI) to a site adjacent to the HRE appears to be required for efficient **transcription** of the MMTV promoter. In JEG-3 choriocarcinoma cells the MMTV promoter is transcribed inefficiently, even after transfection of the receptor cDNA and **treatment** with glucocorticoids or progestins. These cells contain low levels of NFI as cotransfection of NFI cDNA enhances MMTV **transcription** and this effect is inhibited by mutation of the NFI **binding site**. In DNA **binding** experiments with purified NFI from pig liver, the glucocorticoid and progesterone receptors do not co-operate but rather **compete** with NFI for **binding** to their respective sites on the LTR. Similar results are obtained with a functional recombinant NFI synthesized in vitro. **Competition** for DNA **binding** is probably due to steric hindrance as the DNase I footprints of the hormone receptors and NFI do overlap. These results suggest that, though NFI acts as a **transcription factor** on the MMTV promoter, transcriptional activation does not take place through a direct facilitation of DNA **binding** of NFI by steroid hormone receptors.

11/3,AB/30 (Item 30 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

06582830 90280448 PMID: 2191300

Interaction of an NF-kappa B-like **factor** with a site upstream of the c-myc promoter.

Duyao M P; Buckler A J; Sonenshein G E

Department of Biochemistry, Boston University School of Medicine, MA 02118.

Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Jun 1990, 87 (12) p4727-31, ISSN 0027-8424 Journal Code: 7505876

Contract/Grant No.: CA36355; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The c-myc protooncogene has been implicated in control of growth and differentiation of mammalian cells. For instance, growth arrest is often preceded by reduction in c-myc mRNA and gene **transcription**. To elucidate the mechanisms of control of c-myc gene **transcription**, we have begun to characterize the interaction of nuclear **factors** with the 719-base-pair (bp) c-myc regulatory domain, located 1139-421 bp upstream of the P1 start site of the mouse gene. Nuclear extracts from exponentially growing WEHI 231 murine B-lymphoma cells formed multiple complexes in mobility-shift assays. Changes in complex distribution were observed in growth-arrested WEHI 231 cells, and a major site of this interaction mapped to a 21-bp sequence that is similar to the sequences

recognized by the NF-kappa B family of proteins. **Binding** of NF-kappa B-like **factors** was demonstrated by **oligonucleotide competition**. Induction of complex formation upon 70Z/3 pre-B- to B-cell differentiation, enhancement of **binding** by GTP, and detergent-induced release of inhibitor protein suggested that NF-kappa B itself is one member of the family that can **bind**. Transfection of thymidine kinase-chloramphenicol acetyltransferase constructs containing the 21-bp c-myc sequence into Jurkat cells demonstrated increased chloramphenicol acetyltransferase activity upon phorbol ester and phytohemagglutinin **treatment**. These results suggest the involvement of NF-kappa B-like **factors** in the regulation of c-myc **transcription**.

11/3,AB/31 (Item 31 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

06559157 90258885 PMID: 2342464

A novel tumor necrosis **factor**-responsive **transcription factor** which recognizes a regulatory element in hemopoietic growth **factor** genes.

Shannon M F; Pell L M; Lenardo M J; Kuczek E S; Occhiodoro F S; Dunn S M; Vadas M A

Division of Human Immunology, Institute of Medical and Veterinary Science, Adelaide, South Australia.

Molecular and cellular biology (UNITED STATES) Jun 1990, 10 (6)

p2950-9, ISSN 0270-7306 Journal Code: 8109087

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

A conserved DNA sequence element, termed cytokine 1 (CK-1), is found in the promoter regions of many hemopoietic growth **factor** (HGF) genes. Mutational analyses and modification interference experiments show that this sequence specifically **binds** a nuclear **transcription factor**, NF-GMa, which is a protein with a molecular mass of 43 kilodaltons. It interacts with different affinities with the CK-1-like sequence from a number of HGF genes, including granulocyte macrophage colony-stimulating **factor** (GM-CSF), granulocyte (G)-CSF, interleukin 3 (IL-3), and IL-5. We show here that the level of NF-GMa **binding** is induced in embryonic fibroblasts by tumor necrosis **factor**-alpha (TNF-alpha) **treatment** and that the CK-1 sequence from the G-CSF gene is a TNF-alpha-responsive enhancer in these cells. The NF-GMa protein is distinct from another TNF-alpha-responsive **transcription factor**, NF-kappa B, by several criteria. Firstly, several NF-kappa B-**binding sites**, although having sequence similarity with the CK-1 sequence, cannot **compete** efficiently for NF-GMa **binding** to CK-1. Secondly, the CK-1 sequence from both G-CSF and GM-CSF does not respond to phorbol ester **treatment** as would an NF-kappa B-**binding** element. These results demonstrate that NF-GMa is a novel **transcription factor** inducible by TNF-alpha and **binds** to a common element in HGF gene promoters.

11/3,AB/32 (Item 32 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

06381332 90076147 PMID: 2556267

NF-kappa B activation of the cytomegalovirus enhancer is mediated by a viral transactivator and by T cell stimulation.

Sambucetti L C; Cherrington J M; Wilkinson G W; Mocarski E S

Department of Microbiology and Immunology, Stanford University School of Medicine, CA 94305.

EMBO journal (ENGLAND) Dec 20 1989, 8 (13) p4251-8, ISSN

0261-4189 Journal Code: 8208664

Contract/Grant No.: AI07328; AI; NIAID; AI20211; AI; NIAID; HL33811; HL; NHLBI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The expression of cytomegalovirus alpha (immediate early) genes is under control of an enhancer that carries signals for strong constitutive expression as well as response elements for transactivation by viral proteins. We have used synthetic **oligonucleotides** representing the 16, 18 and 19 bp repeat elements within the enhancer to investigate the role of virus-induced cellular **transcription factors** in enhancer activation. We show that the **transcription factor**

NF-kappa B, which **binds** to the 18 bp repeat, plays a central role in enhancer activation in infected human fibroblasts and that activation is mediated by the product of the viral gene iel. The simian immunodeficiency virus kappa B site can functionally substitute for the 18 bp element in transient transactivation assays and can also **compete** efficiently for specific **binding** to the 18 bp repeat element in vitro. Point mutations in the NF-kappa B site within the 18 bp element disrupt iel-mediated transactivation and **binding**. We have found that the characteristics of the 18 bp **binding factor** from human fibroblasts are indistinguishable from NF-kappa B induced by phorbol ester plus mitogen **treatment** of T lymphocytes, as determined by gel mobility shift assay as well as protection of the **binding site** from chemical cleavage. Furthermore, T cell stimulation mediates activation of the viral enhancer via kappa B sites, an observation that may be important in the interaction of cytomegalovirus with the naturally infected human host. Thus, NF-kappa B plays a central role as a target for enhancer activation via viral and cellular **factors**.

11/3,AB/33 (Item 33 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

06319305 90014771 PMID: 2477682

A lipopolysaccharide-induced DNA-**binding** protein for a class II gene in B cells is distinct from NF-kappa B.

Gravallese E M; Boothby M R; Smas C M; Glimcher L H

Department of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts.

Molecular and cellular biology (UNITED STATES) Aug 1989, 9 (8)

p3184-92, ISSN 0270-7306 Journal Code: 8109087

Contract/Grant No.: GM36864; GM; NIGMS

Erratum in Mol Cell Biol 1991 Dec;11(12) 6343

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Class II (Ia) major histocompatibility complex molecules are cell surface proteins normally expressed by a limited subset of cells of the immune system. These molecules regulate the activation of T cells and are required for the presentation of antigens and the initiation of immune responses. The expression of Ia in B cells is determined by both the developmental stage of the B cell and by certain external stimuli. It has been demonstrated previously that **treatment** of B cells with lipopolysaccharide (LPS) results in increased surface expression of Ia protein. However, we have confirmed that LPS **treatment** results in a significant decrease in mRNA encoding the Ia proteins which persists for at least 18 h. Within the upstream regulatory region of A alpha k, an NF-kappa B-like **binding site** is present. We have identified an LPS-induced DNA-**binding** protein in extracts from athymic mice whose spleens consist predominantly of B cells. **Binding** activity is present

? s py<1998 and transcription and factor?

Processing

20949924 PY<1998

370018 TRANSCRIPTION

2977619 FACTOR?

S1 84600 PY<1998 AND TRANSCRIPTION AND FACTOR?

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84600 S1

92687 OLIGONUCLEOTID?

S2 4529 S1 AND OLIGONUCLEOTID?

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4529 S2

1241867 BIND?

S3 3397 S2 AND BIND?

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3397 S3

1161016 BINDING

1203263 SITE?

311820 BINDING(W)SITE?

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374749 OPTIM?

S5 47 S4 AND OPTIM?

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S6 36 RD (unique items)

? t s6/3,ab/all

6/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09549945 97448855 PMID: 9303180

Design of new reagents on the base of DNA duplexes for irreversible inhibition of **transcription factor** NF-kappa B.

Kozlov I A; Kubareva E A; Ivanovskaya M G; Shabarova Z A

Department of Chemistry, Moscow State University, Russia.

Antisense & nucleic acid drug development (UNITED STATES) Aug 1997, 7 (4) p279-89, ISSN 1087-2906 Journal Code: 9606142

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The main purpose of the present work is to search for the **optimal** design of a DNA duplex containing an active group for crosslinking and irreversible inhibition of the **transcription factor** NF-kappa B.

Modified DNA duplexes with an identical nucleotide sequence but different internucleotide phosphates replaced by the trisubstituted pyrophosphate internucleotide group were synthesized. Crosslinking of the human NF-kappa B p50 subunit with the modified DNA duplexes was carried out. It was shown that only four modified duplexes crosslinked with the NF-kappa B p50 subunit. The specificity of these reactions was confirmed. A position of the phosphate in the NF-kappa B recognition site was found where replacement on the active trisubstituted pyrophosphate group resulted in a 50% yield of crosslinking. The fact that DNA duplexes containing the trisubstituted pyrophosphate group specifically react with the NF-kappa B p50 subunit in the Escherichia coli total lysate supports the idea that such modified DNA can be used as high specific inhibitors for DNA-recognizing proteins.

1991

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Set	Items	Description
S1	84600	PY<1998 AND TRANSCRIPTION AND FACTOR?
S2	4529	S1 AND OLIGONUCLEOTID?
S3	3397	S2 AND BIND?
S4	1566	S3 AND (BINDING (W) SITE?)
S5	47	S4 AND OPTIM?
S6	36	RD (unique items)

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1566 S4

367386 COMPET?

S7 491 S4 AND COMPET?

? s s7 and vector?

491 S7

213466 VECTOR?

S8 32 S7 AND VECTOR?

? rd

...completed examining records

S9 20 RD (unique items)

? t s9/3,ab/all

9/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09336346 97234851 PMID: 9173885

Transcriptional activation of the minimal human Proalpha1(I) collagen promoter: obligatory requirement for Sp1.

Poppleton H M; Raghov R

Department of Pharmacology, College of Medicine, The University of Tennessee, Memphis, TN 38104, USA.

Biochemical journal (ENGLAND) Apr 1 1997, 323 (Pt 1) p225-31,

ISSN 0264-6021 Journal Code: 2984726R

Contract/Grant No.: P50AR 39166; AR; NIAMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

A construct containing human Proalpha1(I) collagen gene promoter/enhancer-driven chloramphenicol acetyltransferase (CAT), pCOL-KT, failed to be expressed significantly in Sp1-deficient Schneider Drosophila line 2 (SL2) cells. However, CAT expression was induced 200-fold in SL2 cells co-transfected with pCOL-KT and pPACSp1, an Sp1-expression vector driven by the Drosophila actin 5C promoter. Elimination of the four potential Sp1-binding sites from pCOL-KT (pCOL-KTDeltaI), by removal of the first intron, did not abrogate Sp1-mediated induction of CAT. Even more significantly, a minimal Proalpha1(I) collagen promoter (-100 to +117 bp), containing a TATA box (-28 to -25 bp) and one putative Sp1-binding site (-87 to -82 bp), elicited strong Sp1-induced transactivation. Furthermore, mutation of the Sp1 motif in the minimal Proalpha1(I) collagen promoter-CAT construct abolished Sp1-induced expression of the reporter gene. Purified Sp1 protein bound specifically to DNA fragments of the Proalpha1(I) minimal promoter encompassing the putative Sp1-binding site; Sp1 binding could be competed out by a double-stranded oligonucleotide containing the wild-type Sp1 sequence, while an oligonucleotide containing a mutated Sp1 site failed to compete. Based on these results, we postulate that Sp1 plays an obligatory role in the transcriptional activation of the human Proalpha1(I) collagen gene. Additionally, we propose that a bona fide Sp1 motif, located most proximal to the TATA box, is necessary and sufficient for Sp1-mediated activation of the minimal Proalpha1(I) collagen promoter.

9/3,AB/2 (Item 2 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08584433 95341279 PMID: 7616204

Transcription factor AP-2 regulates expression of the dopamine beta-hydroxylase gene.

Greco D; Zellmer E; Zhang Z; Lewis E
Department of Molecular and Medical Genetics, Oregon Health Sciences University, Portland 97201, USA.

Journal of neurochemistry (UNITED STATES) Aug 1995, 65 (2)
p510-6, ISSN 0022-3042 Journal Code: 2985190R

Contract/Grant No.: GM38696; GM; NIGMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Expression of the gene encoding the neurotransmitter biosynthetic enzyme dopamine beta-hydroxylase (DBH) is regulated in a tissue-specific pattern, and **transcription** is influenced by environmental stimuli. Using the promoter proximal region of the rat DBH gene and nuclear extracts from SHSY-5Y neuroblastoma cells, a DNA-protein complex was identified that is **competitive** with **oligonucleotides** containing the recognition site of **transcription factor** AP-2. DNase footprint analysis identified an AP-2 **binding site** between -136 and -115 of the DBH promoter. Mutation of that AP-2 site results in a sevenfold reduction of basal reporter gene expression, but second messenger-stimulated activity is retained. Cotransfection of an AP-2 expression **vector** and a DBH promoter-reporter construct into cultured cells results in a sixfold stimulation of reporter gene expression, demonstrating the ability of AP-2 to trans-activate the DBH promoter. These results identify a new regulatory element on the rat DBH gene and suggest that the AP-2 site plays a role in maintaining basal levels of DBH **transcription**.

9/3,AB/3 (Item 3 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08512865 95271007 PMID: 7751652

A nuclear **factor** of activated T cell-like **transcription factor** in mast cells is involved in IL-5 gene regulation after IgE plus antigen stimulation.

Prieschl E E; Gouilleux-Gruart V; Walker C; Harrer N E; Baumruker T
Department of Immunodermatology, Sandoz Forschungsinstitut, Vienna, Austria.

Journal of immunology (Baltimore, Md. : 1950) (UNITED STATES) Jun 1 1995, 154 (11) p6112-9, ISSN 0022-1767 Journal Code: 2985117R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

IL-5, which is produced mainly by activated T cells and allergically triggered mast cells, is a major survival and differentiation **factor** for eosinophils, and therefore, is of relevance to diseases associated with this type of cell infiltration, most importantly asthma. In this study, we have examined the transcriptional regulation of human IL-5 in a mouse mast cell line, CPII, stimulated with IgE and Ag. We report that an inducible activity in the region between -177 and -80, and a constitutive activity between -80 and -70, in the promoter of the human gene, are both necessary for the allergically triggered activation. A computer-assisted search for **transcription factor binding** motifs revealed a nuclear **factor** of activated T cell (NF-AT) and a GATA consensus site in the two regions. Corresponding **binding** activities were detected to be present in nuclear extracts from the mouse mast cell line by defined NF-AT

and GATA binding sites as probes for a gel shift analysis. Competition analysis, in combination with probes from the human IL-5 promoter, confirmed that these factors indeed bind to the consensus sequences identified by computer analysis. An oligonucleotide spanning the IL-5 NF-AT consensus site is shown to confer allergic stimulation to a basal IL-5 promoter only in conjunction with the GATA site downstream, indicating that an inducible NF-AT-like factor cooperates with a constitutive member of the GATA transcription factor family in mediating the allergic stimulation of the human IL-5 gene.

9/3,AB/4 (Item 4 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08206483 94342323 PMID: 8063775

Regulation of parathyroid hormone-related protein (PTHrP) gene expression. Sp1 binds through an inverted CACCC motif and regulates promoter activity in cooperation with Ets1.

Dittmer J; Gegonne A; Gitlin S D; Ghysdael J; Brady J N
Laboratory of Molecular Virology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892.

Journal of biological chemistry (UNITED STATES) Aug 26 1994, 269

(34) p21428-34, ISSN 0021-9258 Journal Code: 2985121R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

We have previously shown that mutations in the GGAA core motif of the Ets1 binding site, EBSI, or deletion of EBSI, reduced basal and Tax1 transactivation of the PTHrP P2 promoter. Here we demonstrate that, in addition to EBSI, a CACCC-like motif located between -53 and -58 is required for full basal activity of this promoter in Jurkat T-cells. Site-specific mutations in the CACCC motif decreased promoter activity approximately 5-fold. In an effort to identify transcription factors that bind to the CACCC element, we found that purified human Sp1, as well as Sp1 in HeLa nuclear extract, can specifically bind to a DNA probe that corresponds to the PTHrP-specific sequence between -94 and -34. Gel shift competition studies and DNase I footprinting analyses revealed that Sp1 specifically interacts with the CACCC motif. In the presence of Ets1, the mobility of the Sp1-specific gel shift complex with the PTHrP DNA decreased. DNase I footprint analysis of this gel shift complex showed an extended footprint over both the Sp1 and the Ets1 binding site, demonstrating that Sp1 and Ets1 form a ternary complex with the PTHrP DNA. Cotransfection of an Ets1 and Sp1 expression vector into Drosophila Schneider cells demonstrated that Sp1 can functionally cooperate with Ets1 to transactivate the PTHrP promoter. We conclude from these data that Ets1 and Sp1 can cooperatively regulate PTHrP P2 promoter activity.

9/3,AB/5 (Item 5 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08184799 94321434 PMID: 8045940

Identification of a DNA sequence involved in osteoblast-specific gene expression via interaction with helix-loop-helix (HLH)-type transcription factors.

Tamura M; Noda M

Department of Molecular Pharmacology, Tokyo Medical and Dental University, Japan.

Journal of cell biology (UNITED STATES) Aug 1994, 126 (3)

p773-82, ISSN 0021-9525 Journal Code: 0375356

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

To elucidate regulatory mechanism(s) underlying differentiation of osteoblasts, we examined involvement of helix-loop-helix (HLH)-type **transcription factors** in osteoblast-specific expression of a phenotypic marker gene which encodes osteocalcin, a major noncollagenous bone matrix protein, exclusively expressed in osteoblasts. Overexpression of a dominant negative HLH protein, Id-1, decreased the activity of the 1.1-kb osteocalcin gene promoter cotransfected into rat osteoblastic osteosarcoma ROS17/2.8 cells. Analysis of deletion mutants revealed that a 264-bp fragment of osteocalcin promoter (-198 to +66) was sufficient for the Id-1-dependent suppression. Furthermore, the activity of the same promoter fragment (-198 to +66) was enhanced when antisense Id-1 expression **vector** was cotransfected. This osteocalcin gene promoter region contains two sites of an E-box motif, a consensus **binding site** for HLH proteins, which we refer to as OCE1 (CACATG, at -102) and OCE2 (CAGCTG, at -149), respectively. Mutagenesis in OCE1 but not OCE2 led to greater than 50% reduction in transcriptional activity of the osteocalcin gene promoter. Electrophoresis mobility shift assay indicated that **factors** in nuclear extracts prepared from ROS17/2.8 cells bound to the 30-bp **oligonucleotide** probe containing the E-box motif of OCE1. This **binding** was **competed** out by OCE1 **oligonucleotide** but neither by OCE1 **oligonucleotide** in which E-box motif was mutated nor by OCE2. The OCE1-**binding** activity in the nuclear extracts of ROS17/2.8 cells was reduced by 70% when bacterially expressed Id-1 protein was added to the reaction mixture, suggesting the involvement of HLH proteins in the DNA/protein complex formation. In contrast to the osteoblast-like cells, OCE1-**binding** activity in the nuclear extracts of C3H10T1/2 fibroblasts was very low. However, when these fibroblasts were treated with recombinant human bone morphogenetic protein-2 which induced expression of osteocalcin as well as other phenotypic markers of osteoblasts, OCE1-**binding** activity was increased approximately 40-fold, indicating that OCE1 would be involved in the tissue-specific expression of the osteocalcin gene. These findings indicated for the first time that osteoblast-specific gene **transcription** is regulated via the interaction between certain E-box **binding transcription factor(s)** in osteoblasts and the OCE1 sequence in the promoter region of the osteocalcin gene.

9/3,AB/6 (Item 6 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08155343 94292803 PMID: 8021508

The murine Ig 3' alpha enhancer is a target site with repressor function for the B cell lineage-specific **transcription factor** BSAP (NF-HB, S alpha-BP).

Neurath M F; Strober W; Wakatsuki Y

Mucosal Immunity Section, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892.

Journal of immunology (Baltimore, Md. : 1950) (UNITED STATES) Jul 15 1994, 153 (2) p730-42, ISSN 0022-1767 Journal Code: 2985117R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Using electrophoretic mobility shift assays (EMSAs) we have identified several target sites for nuclear proteins in the murine heavy chain Ig 3' alpha enhancer. Two of these sites, denoted oligo-H and oligo-K, were shown by several criteria, including cell distribution and stimulation experiments, EMSA cross-**competition** studies, and proteolytic clipping bandshift assays, to **bind** to the same protein identical to the **transcription factor** B cell lineage-specific activator protein

(BSAP) (NF-HB, S alpha-BP). To assess the possible functional role of these BSAP **binding sites** in the 3' alpha enhancer, we transiently transfected a construct containing a 314-bp 3' alpha enhancer fragment upstream of a luciferase reporter gene in MOPC-315 cells, a plasmacytoma line lacking BSAP. In these cells, co-transfection with a **vector** expressing recombinant BSAP led to significant reduction in the activity of the 3' alpha enhancer fragment. Conversely, in the mature B lymphoma cell line CH12.LX, a cell line that expresses BSAP and has a less active 3' alpha enhancer, selective BSAP down-regulation by an antisense phosphorothioate **oligonucleotide** was sufficient to considerably up-regulate 3' alpha enhancer activity, as were mutations of both **binding sites** that prevented binding of BSAP to the 3' alpha enhancer. Our findings thus suggest that the natural loss of BSAP expression in terminally differentiated plasma cells contributes to the activation of the murine Ig 3' alpha enhancer.

9/3,AB/7 (Item 7 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08046899 94192856 PMID: 8143900

Characterization of a corticotropin releasing hormone responsive region in the murine proopiomelanocortin gene.

Bishop J F; Mouradian M M

Experimental Therapeutics Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892.

Molecular and cellular endocrinology (IRELAND) Nov 1993, 97

(1-2) p165-71, ISSN 0303-7207 Journal Code: 7500844

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The most potent, physiologic activator of proopiomelanocortin (POMC) gene **transcription** is corticotropin releasing hormone (CRH) and increased intracellular cAMP is critical for this effect. The 5'-flanking region of the murine POMC gene has several potential **binding sites** for regulatory proteins. To characterize the region between nucleotides -141 and -106, which includes a TRE-like site and an adjacent AP-2 consensus sequence, and to study its role in signal-**transcription** coupling, gel mobility shift assays and transient expression of CAT chimeras were performed. In transient transfections of AtT-20 cells with pCATp-141/-106, CRH treatment led to significant increases in CAT expression compared with CRH treatment of cells transfected with the enhancerless **vector**. However, no response to direct activation of cAMP dependent protein kinase or protein kinase C was detected. Despite the high homology of the sequence -137/-131 to the consensus AP-1 **binding site** (TRE), the nuclear **factor(s)** in AtT-20 cells **binding** to this region appears to be different than authentic AP-1 since neither a **competitor oligonucleotide** having the authentic TRE sequence nor antibodies against Jun or Fos affected the gel shift pattern of a probe having the -137/-131 sequence. We conclude that the -141 to -106 region of the murine POMC gene contains a functional CRH responsive element and that second messenger systems that transduce the CRH signal to this element do not exert their actions solely through activation of PKA or PKC.

9/3,AB/8 (Item 8 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07622634 93149117 PMID: 1337144

Activating **transcription factor-2** DNA-**binding** activity is stimulated by phosphorylation catalyzed by p42 and p54 microtubule-associated protein kinases.

Abdel-Hafiz H A; Heasley L E; Kyriakis J M; Avruch J; Kroll D J; Johnson

G L; Hoeffler J P

Department of Medicine, University of Colorado School of Medicine, Denver 80262.

Molecular endocrinology (Baltimore, Md.) (UNITED STATES) Dec 1992
6 (12) p2079-89, ISSN 0888-8809 Journal Code: 8801431

Contract/Grant No.: DK-37871; DK; NIDDK; GM-30324; GM; NIGMS; GM-45872; GM; NIGMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Recent studies have detailed the ability of activating **transcription factor -2 (ATF-2)** to mediate adenoviral Ela stimulation of gene expression; however, an endogenous regulator for the transcriptional activity of this protein has not been described. To characterize the regulation of ATF-2 activity, we have expressed full-length and truncated peptides corresponding to various regions of the ATF-2 protein in bacteria and the baculovirus insect cell system. Bacterially expressed truncated (350-505) but not full-length ATF-2, was able to **bind** a consensus CAMP response element-containing **oligonucleotide**, suggesting the N-terminal moiety may serve as a negative regulator of **DNA-binding** activity. In contrast, the full-length ATF-2 protein expressed in Spodoptera frugiperda (Sf9) cells using a recombinant baculovirus was fully **competent to bind**

DNA. Protein phosphatase 2A reversed the **DNA-binding** activity by dephosphorylating the ATF-2 polypeptide. Microtubule-associated protein kinase catalyzed the phosphorylation and stimulated the **DNA-binding** activity of bacterially expressed full-length ATF-2. Phosphopeptide mapping of phosphorylated ATF-2 proteins identified a single peptide in the N-terminal moiety of ATF-2 phosphorylated by p42 or p54 microtubule-associated protein kinase. Therefore, we propose that phosphorylation of this regulatory site is sufficient to induce an allosteric structural change in the ATF-2 protein, which allows dimerization and subsequent **DNA binding**.

9/3,AB/9 (Item 9 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07612385 93124541 PMID: 8380454

NF-IL6 represses early gene expression of human papillomavirus type 16 through **binding** to the noncoding region.

Kyo S; Inoue M; Nishio Y; Nakanishi K; Akira S; Inoue H; Yutsudo M; Tanizawa O; Hakura A

Department of Obstetrics and Gynecology, Osaka University Medical School, Japan.

Journal of virology (UNITED STATES) Feb 1993, 67 (2) p1058-66,
ISSN 0022-538X Journal Code: 0113724

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The expression of human papillomavirus type 16 (HPV16) early genes, including E6 and E7 transforming genes, is regulated by several cellular **factors binding** to the noncoding region (NCR), such as the glucocorticoid receptor, NF-I, and AP1, all of which are positive regulators. We demonstrated that the nuclear **factor** for interleukin 6 expression (NF-IL6) specifically **binds** to the HPV16 NCR ranging from nucleotides 7007 to 7766 and represses the early gene expression of HPV16. The responsive element in HPV16 NCR was determined within the region ranging from nucleotides 7454 to 7766. In this region, many **binding sites** for other cellular transactivators, such as NF-I and AP1, have been detected. Interestingly, three of seven **binding sites** for NF-I and two of two **binding sites** for AP1 in this region

overlap with the putative NF-IL6 binding sites identified by computer analysis. Competition experiments with the oligonucleotides containing such NF-I or AP1 sites indicated that NF-IL6 certainly binds to them. Furthermore, in a chloramphenicol acetyltransferase assay using mutant NF-IL6 expression vectors, the DNA binding domain of NF-IL6 was shown to be necessary for repression, whereas the functional domain was not. These findings indicate that repression may be caused by competition with other transcriptional activators, such as NF-I and AP1. Thus, NF-IL6 may play a significant role in the regulation of viral transcription as a part of the host's resistance to viral infection.

9/3,AB/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07551153 93077573 PMID: 1332964

Characterization of a minimal promoter element required for transcription of the mouse type II beta regulatory subunit (RII beta) of cAMP-dependent protein kinase.

Luo Z; Singh I S; Fujihira T; Erlichman J
Department of Medicine, Albert Einstein College of Medicine, Bronx, New York 10461.

Journal of biological chemistry (UNITED STATES) Dec 5 1992, 267
(34) p24738-47, ISSN 0021-9258 Journal Code: 2985121R

Contract/Grant No.: DK-27736; DK; NIDDK

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The 5'-flanking DNA of the mouse RII beta subunit of the cAMP-dependent protein kinase gene was characterized by transient transfection of RII beta-CAT constructs into mouse neuroblastoma cells (NB2a) and Chinese hamster ovary (CHO) cells and by gel mobility shift and footprinting assays. The minimal promoter of the RII beta gene was composed of two adjacent functional elements. A 3'-element which supported enhanced CAT activity was located between base pairs (bp) -267/-168 from the translation initiation start site. CAT plasmids containing these RII beta sequences showed 12- and 16-fold increased CAT activity in the NB2a and CHO cells, respectively, compared to the basic CAT vector. Plasmids containing 20 additional bp 5' to the -267/-168 fragment showed 2-fold more CAT activity than the shorter fragment in NB2a cells, while CAT activity in CHO cells was nearly the same for both constructs. CAT plasmids containing only this 20-bp fragment showed 9- and 13-fold increased CAT activity in NB2a and CHO cells, respectively. The core promoter of the RII beta gene lacked classical TATA and CAT sequences, but contained 3 copies of the Sp1 core consensus sequence. Gel mobility shift assays using 32P-labeled 5'-flanking DNA containing bp -291/-49 and nuclear extracts from NB2a and CHO cells displayed several retarded bands in the gels suggesting complex formation with nuclear DNA-binding factors. Unlabeled DNA containing bp -291/-49 blocked the appearance of all retarded bands. Competition using an oligonucleotide corresponding to the Sp1 DNA-binding site effectively blocked the appearance of the two more slowly migrating bands but did not affect the major rapidly migrating bands. DNase I footprinting analysis using purified Sp1 protein confirmed that Sp1 could bind to the Sp1 sites. Methylation interference and mutational analysis showed that one of the faster migrating bands was the result of factor binding to the DNA sequence adjacent to the Sp1 sites. Additional tissue-specific nuclear-binding factor sequences were detected upstream of the core promoter. Our data suggest that the core promoter of the RII beta gene can initiate transcription from the DNA around the Sp1 sites but that there are tissue-specific nuclear factor-binding sites located distal to the Sp1 sites.

9/3,AB/11 (Item 11 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07334400 92264735 PMID: 1586166

The first 22 base pairs of the proximal promoter of the rat class I alcohol dehydrogenase gene is bipartite and interacts with multiple DNA-binding proteins.

Potter J J; Mezey E; Cornelius P; Crabb D W; Yang V W
Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

Archives of biochemistry and biophysics (UNITED STATES) Jun 1992,

295 (2) p360-8, ISSN 0003-9861 Journal Code: 0372430

Contract/Grant No.: AA00626; AA; NIAAA; AA06434; AA; NIAAA

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The rat class I alcohol dehydrogenase (ADH) gene is primarily expressed in the liver. We previously showed that the liver-enriched **transcription factor**, the CCAAT/enhancer binding protein (C/EBP), binds to the proximal promoter of the rat class I ADH gene between positions -11 and -22 relative to the start site of **transcription**. We now demonstrate that another **transcription factor**, the liver activator protein (LAP), also interacts with the same region of the promoter based on the following observations: (1) LAP synthesized by in vitro **transcription** and translation of cloned cDNA sequence forms complexes with an **oligonucleotide** containing the C/EBP-binding sequence within the ADH promoter as determined by the electrophoretic mobility shift assay (EMSA), (2) purified LAP interacts with the proximal ADH promoter when analyzed by the DNase I protection assay, and (3) an ADH promoter-reporter gene construct containing the C/EBP-binding site is transactivated by an eukaryotic expression vector containing the LAP sequence. EMSA of an **oligonucleotide** containing the first 22 base pairs (between positions -1 and -22) of the ADH promoter with rat liver nuclear extracts (RLNE) resulted in the formation of two major complexes. Complex 1 was competed away by a heterologous **oligonucleotide** containing a C/EBP-binding site within the promoter of the adipocyte 422 (aP2) gene, while complex 2 was not. Additional **competition** experiments with the ADH or 422 (aP2) **oligonucleotide** using either RLNE or extracts from 3T3-L1 adipocytes demonstrated that complex 1 contains either C/EBP or LAP, while complex 2 contains a DNA-binding protein that binds to a novel sequence 5'-TGGCCCAGTT-3' between positions -1 and -10 of the ADH promoter. Ultraviolet cross-linking between RLNE and a labeled **oligonucleotide** containing the above sequence indicates that this protein, designated EDBP (for enhancer-site downstream binding protein), has an estimated molecular weight of 47 kDa, which is larger than that reported for either C/EBP (42 kDa) or LAP (36 kDa).

9/3,AB/12 (Item 12 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07167274 92085393 PMID: 1727489

The cellular proto-oncogene product Myb acts as transcriptional activator of the long terminal repeat of human T-lymphotropic virus type I.

Dasgupta P; Reddy C D; Saikumar P; Reddy E P
Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104-4268.

Journal of virology (UNITED STATES) Jan 1992, 66 (1) p270-6,

ISSN 0022-538X Journal Code: 0113724

Contract/Grant No.: AI 25380; AI; NIAID; CA 10815; CA; NCI; CA 55492; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The proto-oncogene c-myb encodes a nuclear **transcription factor** that **binds** to DNA in a sequence-specific manner and activates **transcription** of several viral and cellular genes. Expression of the c-myb gene is induced in mitogen- and/or antigen-stimulated T lymphocytes, which are also the preferential target cells of human T-lymphotropic virus type I (HTLV-I) in vivo and in vitro. We report here that Myb **binds** to the HTLV-I long terminal repeat (LTR) in four different regions in a sequence-specific manner. Electrophoretic mobility shift assay using labeled LTR fragments as well as labeled double-stranded **oligonucleotides** show that there are two high-affinity and two low-affinity Myb-**binding sites** present in the HTLV-I LTR. DNase I footprinting analysis and **oligonucleotide competition** experiments indicate that this **binding** is sequence specific. Cotransfection experiments in HeLa cells, using a Myb expression **vector** and chloramphenicol acetyltransferase reporter gene linked to the HTLV-I LTR, show that Myb activates HTLV-I LTR-mediated **transcription** by a **factor** of four-to sixfold. Thus, in HTLV-I-infected T cells, Myb protein **binding** to the HTLV-I LTR may constitute one of the signal that regulate HTLV-I **transcription** in vivo.

9/3,AB/13 (Item 13 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07076105 92011403 PMID: 1917865

Participation of ABF-1 protein in expression of the Saccharomyces cerevisiae CAR1 gene.

Kovari L Z; Cooper T G
Department of Microbiology and Immunology, University of Tennessee, Memphis 38163.

Journal of bacteriology (UNITED STATES) Oct 1991, 173 (20)
p6332-8, ISSN 0021-9193 Journal Code: 2985120R

Contract/Grant No.: GM35642; GM; NIGMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

DNA fragments previously shown to be required for expression of the CAR1 (arginase) gene in Saccharomyces cerevisiae and to support transcriptional activation of a reporter gene in a heterologous expression **vector** were shown to **bind** purified regulatory protein ABF-1. Two ABF-1 sites were identified in the CAR1 upstream region, one to which ABF-1 protein bound with high affinity and a second to which it bound much less avidly. The higher-affinity ABF-1 **binding site** upstream of CAR1 was an effective **competitor** of the HMRE, ARS1 B domain, and COR2-GFI **binding** sequences for protein **binding**. Point mutations in the CAR1 high-affinity ABF-1 **binding site** resulted in a 12-fold loss of transcriptional activation of a reporter gene compared with the wild-type CAR1 DNA fragment. These data are consistent with the suggestion that ABF-1 protein is one of the **transcription factors** involved in expression of the CAR1 gene.

9/3,AB/14 (Item 14 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07023524 91332075 PMID: 1869565

The upstream stimulatory **factor binds** to and activates the promoter of the rat class I alcohol dehydrogenase gene.

Potter J J; Cheneval D; Dang C V; Resar L M; Mezey E; Yang V W
Department of Medicine, Johns Hopkins University School of Medicine,
Baltimore, Maryland 21205.

Journal of biological chemistry (UNITED STATES) Aug 15 1991, 266

(23) p15457-63, ISSN 0021-9258 Journal Code: 2985121R

Contract/Grant No.: AA000626; AA; NIAAA; CA51497; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The gene encoding rat class I alcohol dehydrogenase (ADH) is expressed primarily in the liver. Recent studies in our laboratories indicate that multiple cellular **factors** present in the rat liver interact with various regions of the promoter of this gene. One of the regions contains the sequence 5'-CACATG-3' that has an "E box" homology to which a number of **transcription factors** containing the basic helix-loop-helix motif **bind**. We now demonstrate that the human **transcription factor**, upstream stimulatory **factor** (USF), a basic helix-loop-helix-containing protein, **binds** to and activates the promoter of the rat class I ADH gene. Electrophoretic mobility shift assays of labeled **oligonucleotide** containing the 5'-CACATG-3' sequence within the ADH promoter revealed the formation of multiple DNA-protein complexes when nuclear extracts obtained from adult rat liver were used. The **binding** of proteins to the DNA could be **competed** away with an **oligonucleotide** specifying a sequence within the adenovirus major late promoter (MLP) that had previously been shown to **bind** USF. Similar complexes were observed when electrophoretic mobility shift assays of labeled MLP **oligonucleotide** were performed with rat liver nuclear extracts. Conversely, nuclear extracts isolated from HeLa cells, cells known to have abundant USF, contain **factors** that interact with the sequence present in the ADH promoter. This interaction could be **competed** efficiently by the MLP **oligonucleotide**. USF synthesized in an in vitro **transcription** and translation system also **binds** to the ADH promoter as well as to the MLP. In addition, antiserum directed against USF recognizes **factors** present in the rat liver nuclear extracts that interact with the ADH promoter. Furthermore, **transcription** directed from both the ADH and the adenovirus major late promoters was inhibited by an **oligonucleotide** representing the USF-binding site within the ADH promoter in a cell-free in vitro **transcription** system. Lastly, an ADH promoter-reporter gene construct was transactivated by an eukaryotic expression **vector** containing USF in HepG2 cells co-transfected with the two constructs. These experiments demonstrate that USF is present in the rat liver and that it **binds** to and activates the promoter of the rat class I ADH gene in a sequence-specific manner.

9/3,AB/15 (Item 15 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

06743525 91056553 PMID: 2243379

The upstream **factor-binding site** is not essential for activation of **transcription** from the adenovirus major late promoter.

Reach M; Babiss L E; Young C S

Department of Microbiology, College of Physicians and Surgeons, Columbia University, New York, New York 10032.

Journal of virology (UNITED STATES) Dec 1990, 64 (12) p5851-60

, ISSN 0022-538X Journal Code: 0113724

Contract/Grant No.: CA13696; CA; NCI; CA48707-10; CA; NCI; GM38125; GM; NIGMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

An adenovirus major late promoter (MLP) has been constructed with a 4-bp alteration in the sequence which **binds the transcription factor** known as USF or MLTF. This upstream element has often been considered necessary and sufficient for maximal **transcription** of the MLP. A duplex **oligonucleotide** containing the mutant sequence was not capable of **binding** specific proteins in a band shift assay, nor was it capable of inhibiting such **binding** by the wild-type sequence. In an in vitro assay, the mutant sequence was incapable of inhibiting **transcription** from a duplex sequence containing the MLP, whereas the wild-type sequence could. These two pieces of evidence suggest that the sequence is functionally impaired. Surprisingly, a virus containing the mutant MLP had a normal replication phenotype. On more detailed examination however, we show that the mutant viral MLP was deficient in **transcription** at 9 h postinfection but that the rate of **transcription** was close to normal by 20 h postinfection. An inverted CAAT box located immediately upstream of the USF-**binding** element was not previously thought to be of importance to the functioning of the MLP. However, a single point mutation in the CAAT box, placed in the USF mutant background, had a marked effect upon **transcription** from the MLP. This result suggests that the MLP may exhibit functional redundancy in which either the USF-**binding site** or the CAAT box can serve as an upstream promoter element. Neither of the mutant viruses displayed any change in the levels of the divergent IVa2 **transcription** unit, suggesting that the levels of divergent **transcription** are not determined by **competition** for limiting **transcription factors**.

9/3,AB/16 (Item 16 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

06734244 91045943 PMID: 2236022

Myb protein **binds** to human immunodeficiency virus 1 long terminal repeat (LTR) sequences and transactivates LTR-mediated **transcription**.

Dasgupta P; Saikumar P; Reddy C D; Reddy E P
Wistar Institute of Anatomy and Biology, Philadelphia, PA 19104.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Oct 1990, 87 (20) p8090-4, ISSN 0027-8424 Journal Code: 7505876
Contract/Grant No.: AI 25380; AI; NIAID; CA 10815; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The protooncogene c-myb encodes a nuclear **transcription factor** that **binds** to DNA in a sequence-specific manner and transactivates **transcription** of several viral and cellular genes. The expression of c-myb is induced in mitogen-stimulated peripheral blood lymphocytes and is constitutively expressed in several CD4+ T-cell and myeloid cell lines, all of which constitute excellent targets for human immunodeficiency virus (HIV) infection and replication. We looked for the presence of Myb-**binding** motifs in human retroviral long terminal repeats (LTRs) and tested for Myb **binding** to HIV-1 LTR sequences by using a highly purified recombinant Myb protein. Our results show that HIV-1 LTR contains one high-affinity Myb-**binding site** along with two or more low-affinity **binding sites**. DNase I protection analysis as well as **oligonucleotide competition** experiments indicate that this **binding** is sequence specific. Introduction of purified Myb protein directly into HeLa cells harboring HIV-1 LTR chloramphenicol acetyltransferase **vectors** indicates that Myb protein transactivates HIV-1 LTR-mediated **transcription**. Thus, Myb protein **binding** to HIV LTR sequences may constitute one of the signals that regulates HIV-1 **transcription**.

9/3,AB/17 (Item 17 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

06618729 90318353 PMID: 2115115

A cis-acting element present in multiple genes serves as a repressor protein **binding site** for the yeast CAR1 gene.

Luche R M; Sumrada R; Cooper T G
Department of Microbiology and Immunology, University of Tennessee,
Memphis 38163.

Molecular and cellular biology (UNITED STATES) Aug 1990, 10 (8)

p3884-95, ISSN 0270-7306 Journal Code: 8109087

Contract/Grant No.: GM-35642; GM; NIGMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Induction of the arginase (CAR1) gene expression in *Saccharomyces cerevisiae* has previously been shown to require participation of a cis-dominantly regulated upstream repression sequence (URS). Deletion of this element results in high-level expression of the CAR1 gene without inducer. To determine the structure of the CAR1 URS element, we performed a saturation mutagenesis. Results of the mutagenic analysis indicated that the CAR1 URS was a 9-base-pair palindromic sequence, 5'-AGCCGCCGA-3'. A DNA fragment containing this sequence was shown to **bind** one or more proteins by a gel shift assay. DNA fragments containing point mutations that completely eliminated URS function were not effective **competitors** in this assay, whereas those which supported URS function were effective **competitors**. Sequences in the 5'-flanking regions of 14 other genes were found to be homologous to the CAR1 URS. These sequences were shown to support varying degrees of URS function in the expression **vector** assay, to **bind** protein as demonstrated by the gel shift assay, and to **compete** with a DNA fragment containing the CAR1 URS for protein **binding**. These results indicate that the CAR1 URS element possesses the characteristics of a repressor **binding site**. Further, they are consistent with the suggestion that sites homologous to the CAR1 URS may be situated in the 5'-flanking regions of multiple unrelated yeast genes. The widespread occurrence of this element raises the possibility that it is the target site for one or more negatively acting general **transcription factors**.

9/3,AB/18 (Item 18 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

06415455 90108682 PMID: 2606350

Differentiation-induced gene expression in 3T3-L1 preadipocytes: CCAAT/enhancer **binding** protein interacts with and activates the promoters of two adipocyte-specific genes.

Christy R J; Yang V W; Ntambi J M; Geiman D E; Landschulz W H; Friedman A D; Nakabeppu Y; Kelly T J; Lane M D

Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

Genes & development (UNITED STATES) Sep 1989, 3 (9) p1323-35,

ISSN 0890-9369 Journal Code: 8711660

Contract/Grant No.: 5F32-NIDDK08088; DK; NIDDK; 5F32-NIDDK081903; DK; NIDDK; NIDDK-38418; DK; NIDDK

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Previous studies have shown that differentiation of 3T3-L1 preadipocytes leads to the transcriptional activation of a group of adipose-specific

genes. As an approach to defining the mechanism responsible for activating the expression of these genes, we investigated the **binding** of nuclear **factors** to the promoters of two differentiation-induced genes, the 422(aP2) and stearoyl-CoA desaturase 1 (SCD1) genes. DNase I footprinting and gel retardation analysis identified two **binding** regions within the promoters of each gene that interact with nuclear **factors** present in differentiated 3T3-L1 adipocytes. One differentiation-induced nuclear **factor** interacts specifically with a single **binding site** in the promoter of each gene. **Competition** experiments showed that the interaction of this nuclear **factor** with the SCD1 promoter was prevented specifically by a synthetic **oligonucleotide** corresponding to the site footprinted in the 422(aP2) promoter. Several lines of evidence indicate that the differentiation-induced nuclear **factor** is CCAAT/enhancer **binding** protein (C/EBP), a DNA-**binding** protein first isolated from rat liver. Bacterially expressed recombinant C/EBP **binds** to the same site at which the differentiation-specific nuclear **factor** interacts within the promoter of each gene. Northern analysis with RNA from 3T3-L1 cells shows that C/EBP mRNA abundance increases markedly during differentiation. Transient cotransfection studies using a C/EBP expression **vector** demonstrate that C/EBP can function as a trans-activator of both the 422(aP2) and SCD1 gene promoters.

9/3,AB/19 (Item 19 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

06373582 90066433 PMID: 2555687

Multiple sequence elements of a single functional class are required for cyclic AMP responsiveness of the mouse c-fos promoter.

Berkowitz L A; Riabowol K T; Gilman M Z

Cold Spring Harbor Laboratory, New York 11724.

Molecular and cellular biology (UNITED STATES) Oct 1989, 9 (10)

p4272-81, ISSN 0270-7306 Journal Code: 8109087

Contract/Grant No.: AI27270; AI; NIAID; CA45642; CA; NCI; CA46370; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Agents that elevate the intracellular concentration of cyclic AMP (cAMP) rapidly and transiently induce expression of the c-fos proto-oncogene in BALB/c 3T3 cells. We show that the mouse c-fos promoter-enhancer region contains multiple elements that contribute to cAMP responsiveness of the promoter in transient expression assays. The most potent element was found to correspond to a previously mapped basal promoter element and protein-**binding site** located 65 base pairs upstream of the transcriptional initiation site. This element and two less potent sites contained a match to the cAMP response element (CRE) core sequence defined in several mammalian genes. The relative potencies of these elements corresponded with their relative affinities for cellular **factors** that bound to the CRE in vitro. Mutation of all three elements failed to abolish completely cAMP responsiveness of the c-fos promoter in the transient expression assay. However, we present evidence that this residual responsiveness may have been due to sequences present in **vector** DNA. Finally, we show, by using a new microinjection **competition** assay, that a double-stranded **oligonucleotide** carrying the major c-fos CRE is sufficient to block induction of the endogenous c-fos gene by cAMP. Therefore, induction of the endogenous gene requires positively acting cellular **factors** that interact with a single functional class of regulatory sites in the c-fos gene. Unrelated regulatory elements, such as the serum response element and putative AP-2 sites, are not by themselves sufficient to mediate the cAMP response.

9/3,AB/20 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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07521676 BIOSIS NO.: 000091084805
TRANSACTIVATION OF THE HUMAN INSULIN RECEPTOR GENE BY THE CAAT-ENHANCER

BINDING PROTEIN

AUTHOR: MCKEON C; PHAM T

AUTHOR ADDRESS: DIABETES BRANCH, NATIONAL INST. DIABETES KIDNEY, BETHESDA,
MD. 20892.

JOURNAL: BIOCHEM BIOPHYS RES COMMUN 174 (2). 1991. 721-728. 1991

FULL JOURNAL NAME: Biochemical and Biophysical Research Communications

CODEN: BBRCA

RECORD TYPE: Abstract

LANGUAGE: ENGLISH

ABSTRACT: Within human insulin receptor gene there are three consensus **binding sites** for the CAAT/enhancer **binding** protein (C/EBP). Two sites are located in the 5'flanking region and the other is in the first intron. We have studied the ability of these sequences to be regulated by C/EBP. A eukaryotic expression **vector** containing these sequences can be transactivated in a dose-dependent manner by a C/EBP expression **vector** when co-transfected into NIH-3T3 cells. In addition, double stranded **oligonucleotides** corresponding to two of these sequences can **bind** C/EBP in a gel retardation assay. These two **oligonucleotides** can **compete** with each other to **bind** C/EBP. These findings suggest that this **transcription factor** may play a role in the regulation of insulin receptor gene expression in vivo.

1991

6/3,AB/2 (Item 2 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09384299 97288385 PMID: 9143363

Effect of acetaldehyde on Sp1 **binding** and activation of the mouse alpha 2(I) collagen promoter.

Miao K; Potter J J; Anania F A; Rennie-Tankersley L; Mezey E
Department of Medicine, Johns Hopkins University School of Medicine,
Baltimore, Maryland 21205-2195, USA.

Archives of biochemistry and biophysics (UNITED STATES) May 1
1997, 341 (1) p140-52, ISSN 0003-9861 Journal Code: 0372430

Contract/Grant No.: AA00626; AA; NIAAA; T32 AA07467; AA; NIAAA

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Acetaldehyde activates the mouse alpha 2(I) collagen promoter and this effect is mediated in part by increased **binding** of nuclear **factor** I (NF-I). Additional mechanisms may exist since deletions in the promoter upstream to the NF-I **binding site** prevented enhancement by acetaldehyde. Three adjacent areas of **binding** by nuclear proteins from activated hepatic stellate cells were identified at -568 to -554 (region 1), -542 to -518 (region 2), and -473 to -453 (region 3) of the promoter using DNase I protection analyses. Multiple DNA-protein complexes were formed in electrophoretic mobility shift assays with **oligonucleotide** probes specifying the three regions. Sp1 and NF-1 bound to all three regions, while Sp3 bound to region 2. Acetaldehyde decreased nuclear protein **binding** to all three regions. Mutations of regions 1, 2, and 3 reduced basal activity of the promoter and inhibited acetaldehyde stimulation in transfected stellate cells. Acetaldehyde inhibited the stimulatory effect of the Sp1 vector pPacSp1 on the promoter in transfected Drosophila cells. In conclusion, three regions of Sp1 **binding** were identified and are required for **optimal** activity of the alpha 2(I) collagen promoter. Sp1 is required for basal activity of the alpha 2(I) collagen promoter; however, the enhancing effect of acetaldehyde on the promoter is not mediated by Sp1.

6/3,AB/3 (Item 3 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09355002 97265371 PMID: 9111308

POU domain **factors** of the Brn-3 class recognize functional DNA elements which are distinctive, symmetrical, and highly conserved in evolution.

Gruber C A; Rhee J M; Gleiberman A; Turner E E
Department of Psychiatry, University of California, San Diego, La Jolla, USA.

Molecular and cellular biology (UNITED STATES) May 1997, 17 (5)
p2391-400, ISSN 0270-7306 Journal Code: 8109087

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

To better understand the diversity of function within the POU domain class of transcriptional regulators, we have determined the **optimal** DNA recognition site of several proteins of the POU-IV (Brn-3) subclass by random **oligonucleotide** selection. The consensus recognition element derived in this study, **ATAATTAAT**, is clearly distinct from octamer sites described for the POU **factor** Oct-1. The **optimal** POU-IV site determined here also **binds** Brn-3.0 with significantly higher affinity than consensus recognition sites previously proposed for this POU subclass. The **binding** affinity of Brn-3.0 on its **optimal** site, several variants of this site, and several naturally occurring POU recognition

elements is highly correlated with the activation of reporter gene expression by Brn-3.0 in transfection assays. The preferred DNA recognition site of Brn-3.0 resembles strongly the **optimal** sites of another mammalian POU-IV class protein, Brn-3.2, and of the *Caenorhabditis elegans* Brn-3.0 homolog Unc-86, demonstrating that the site-specific DNA recognition properties of these **factors** are highly conserved between widely divergent species.

6/3,AB/4 (Item 4 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09261477 97151508 PMID: 8997490

DNA-binding specificity of PAR and C/EBP leucine zipper proteins: a single amino acid substitution in the C/EBP DNA-binding domain confers PAR-like specificity to C/EBP.

Falvey E; Marcacci L; Schibler U
Department of Molecular Biology, University of Geneva, Switzerland.
Biological chemistry (GERMANY) Dec 1996, 377 (12) p797-809,
ISSN 1431-6730 Journal Code: 9700112

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

PAR and C/EBP family proteins are liver-enriched basic leucine zipper (bZip) **transcription factors** that **bind** similar sites on the promoters of albumin and cholesterol 7 alpha hydroxylase genes. However, C/EBP proteins have a more relaxed **binding** specificity than PAR proteins, in that they recognize many sites within promoter or randomly selected rat genomic DNA sequences that are ignored by PAR proteins. Thus, DNase I protection experiments suggest that C/EBP recognizes a **binding site** with an affinity similar to the one of the cholesterol 7 alpha hydroxylase gene promoter every 200 to 300 bp. The frequency of PAR protein **binding sites** with comparable affinities is about 20-fold lower in the rat genome. By using a PCR-based amplification assay we selected high affinity DNA-binding sites for C/EBP beta and the PAR protein DBP from a pool of **oligonucleotides**. Both proteins indeed recognize similar sequences with the **optimal** core, **binding** sequences 5'RTTAY.GTAAY3'. However, as expected, DBP, is considerably less tolerant to deviations from the consensus site. Here we have characterized a single amino acid substitution mutant of C/EBP beta that increases its target site specificity. This protein, C/EBP beta V > A, contains a valine to alanine substitution at position 13 of the basic domain (residue 216 of C/EBP beta). C/EBP beta V > A selectively **binds** only the subset of C/EBP sites that are also DBP sites, both as **oligonucleotides** and within the natural contexts of the albumin and cholesterol hydroxylase promoters.

6/3,AB/5 (Item 5 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09068612 96419171 PMID: 8821944

In vitro selection of **optimal** AbrB-binding sites: comparison to known in vivo sites indicates flexibility in AbrB **binding** and recognition of three-dimensional DNA structures.


Xu K; Strauch M A
Department of Molecular and Experimental Medicine, Scripps Research Institute, La Jolla, California 92037, USA.

Molecular microbiology (ENGLAND) Jan 1996, 19 (1) p145-58,
ISSN 0950-382X Journal Code: 8712028

Contract/Grant No.: GM46700; GM; NIGMS

Document type: Journal Article

Languages: ENGLISH



Main Citation Owner: NLM

Record type: Completed

The AbrB protein of *Bacillus subtilis* regulates expression of numerous genes, primarily through specific **binding** interactions to DNA regions containing transcriptional promoters. Although over 15 target regions for AbrB **binding** to chromosomally located sequences have been analysed by DNase I footprinting, no obvious consensus sequence or motif has yet emerged from their examination. Using in vitro selection techniques, we have isolated **optimal AbrB-binding sites** from **oligonucleotides** containing 22 or 44 random base pairs. The best of these sites have an apparent in vitro Kd which is fivefold lower than a similar-sized DNA fragment containing the sequence corresponding to the **AbrB-binding site** on the *spo0E* gene. We tested one of the sites in vivo and found that it confers AbrB-mediated control upon a promoter not normally regulated by AbrB. In each of four separate trials, the selected sites possess motifs that converge to a simple consensus. It is argued that the nature and spacing of these motifs produce a type of three-dimensional DNA structure recognizable by AbrB, and that known in vivo sites, which lack these motifs, possess an approximation of the **optimal** structural determinant.

6/3,AB/6 (Item 6 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09026114 96394406 PMID: 8798512

Requirement of an upstream AP-1 motif for the constitutive and phorbol ester-inducible expression of the urokinase-type plasminogen activator receptor gene.

Lengyel E; Wang H; Stepp E; Juarez J; Wang Y; Doe W; Pfarr C M; Boyd D
Department of Obstetrics and Gynecology, Technical University of Munich, 81675 Munich, Germany.

Journal of biological chemistry (UNITED STATES) Sep 20 1996, 271

(38) p23176-84, ISSN 0021-9258 Journal Code: 2985121R

Contract/Grant No.: R01CA58311; CA; NCI; R01DE10845; DE; NIDCR

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The urokinase-type plasminogen activator receptor (u-PAR) facilitates extracellular matrix proteolysis by accelerating plasmin formation at the cell surface. The present study was undertaken to identify elements in the u-PAR promoter required for the elevated expression of this **binding site**. Toward this end, we used two cultured colon cancer cell lines; one (RKO) has a transcriptionally activated u-PAR gene, and the other (GEO) overexpresses the receptor only after phorbol ester treatment. A chloramphenicol acetyltransferase (CAT) reporter driven by 398 nucleotides of 5' regulatory sequence of the u-PAR gene was strongly activated in the RKO cells, which displays approximately 3×10^5 receptors/cell. A region of this promoter between -197 and -8 was required for **optimal** expression, as indicated using a CAT reporter driven by 5' deleted fragments. DNase I footprinting revealed three protected regions (I, -190 to -171; II, -148 to -124; and III, -99 to -70) in this part of the promoter. Mutation of an AP-1 **binding site** at -184 within region I reduced activation of the promoter by 85%. Deletion of either region II or III also reduced promoter activity by over 60%. An **oligonucleotide** spanning the AP-1 motif at -184 bound, specifically, nuclear **factors** from RKO cells, and antibodies specific for Jun-D, c-Jun, or Fra-1 proteins supershifted the complex indicating the presence of these proteins. The amount of these **factors** was reduced in GEO cells in which the u-PAR gene is only weakly transcriptionally activated. Expression of a vector encoding a wild-type Jun-D cDNA increased u-PAR promoter activity in GEO cells. Conversely, transfection of RKO cells with a transactivation domain-lacking Jun-D expression construct resulted in a

dose-dependent decrease in u-PAR promoter activity. Treatment of GEO cells with phorbol ester increased u-PAR mRNA and the activity of a CAT reporter driven by the wild-type but not the AP-1 (-184)-mutated u-PAR promoter, and this was associated with a strong induction in the amount of Jun-D, c-Jun, and c-Fos. Methylation interference studies using a fragment of the u-PAR promoter (spanning -201 to -150) bound with nuclear extracted proteins from RKO cells, and phorbol 12-myristate 13-acetate-treated and -untreated GEO cells showed that the contact points corresponded to the AP-1 **binding site** at -184. Thus, the elevated expression of u-PAR in RKO cells, which constitutively produces this **binding site**, as well as in phorbol 12-myristate 13-acetate-stimulated GEO cells requires an AP-1 motif located 184 bp upstream of the transcriptional start site.

6/3,AB/7 (Item 7 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08934849 96290426 PMID: 8704176

Two pathways can activate the interleukin-5 gene and induce **binding** to the conserved lymphokine element 0.

Karlen S; D'Ercole M; Sanderson C J

TVW Telethon Institute for Child Health Research, Perth, Australia.

Blood (UNITED STATES) Jul 1 1996, 88 (1) p211-21, ISSN

0006-4971 Journal Code: 7603509

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Eosinophilia is a uniquely specific phenomenon regulated by interleukin-5 (IL-5), suggesting specific control for IL-5 gene expression. Using a transient-transfection reporter assay and DNA mobility-shift experiments in EL4 mouse lymphoma cells, reporter expression and **binding** of **transcription factors** to the conserved lymphokine element 0 (CLE0) in the mouse (mIL-5) promoter was investigated. Activation of the IL-5 promoter required costimulation of T cells with phorbol ester (phorbol 12-myristate 13-acetate [PMA]) and cyclic adenosine 3',5'-monophosphate (cAMP), but was blocked by the immunosuppressive drug, cyclosporin A (CsA). **Binding** to CLE0 was induced under conditions **optimal** for IL-5 **transcription** but was not blocked by CsA. CD28-induced signals could partly substitute for cAMP. However, the effects of cAMP, but not of CD28, were sensitive to the cAMP-dependent protein kinase inhibitor, H89, suggesting that CD28 does not involve a cAMP mechanism. It therefore appears that IL-5 expression can be induced by at least two distinct stimulatory pathways. Although CLE0 contains sequences similar to AP-1 and NF-AT, only the AP-1 moiety of the CLE0 element could be demonstrated to have inducible **binding**. Experiments with antisera to the AP-1 family of **transcription factors** indicated that c-fos and JunB **bind** to the IL-5 CLE0 in activated lymphoma cells. The role of the NF-AT-like element was less clear. A constitutively expressed protein showed a weak band that was inhibited by mIL-2 NF-AT competitor sequences. However, this protein did not react with an anti-NF-ATp antiserum. On the other hand, **transcription** was partially inhibited by an **oligonucleotide** containing the intact NF-AT-like element from CLE0, suggesting that the element is important for **optimal transcription**, but the nature of the protein **binding** to it remains unknown. The fact that these **factors** are induced in a subclone of EL4 that does not express IL-5 and **bind** to a number of other cytokine gene promoters suggests that although **binding** to CLE0 appears to be necessary for IL-5 **transcription**, other **factors** must control the specific expression of the gene.

6/3,AB/8 (Item 8 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08878667 96226350 PMID: 8649821

ETS1 and ETS2 in p53 regulation: spatial separation of ETS **binding sites** (EBS) modulate protein: DNA interaction.

Venanzoni M C; Robinson L R; Hodge D R; Kola I; Seth A

Laboratory of Molecular Oncology, National Cancer Institute-FCRDC, Frederick, Maryland 21702-1201, USA.

Oncogene (ENGLAND) Mar 21 1996, 12 (6) p1199-1204, ISSN 0950-9232 Journal Code: 8711562

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

p53 is an extensively studied tumor suppressor gene implicated in the genesis of a large number of varied tumors. However, the pathways of regulation for the wild-type p53 gene and its product are as yet unknown. In situ hybridization analyses of ETS1 and ETS2 expression during mouse embryogenesis, have shown a pattern similar to that of p53 gene expression. Significantly, we have identified several ETS-**binding sites** (EBS) in the promoter regions of the human and mouse p53 genes. In the human promoter two of these EBS are present in the form of a palindrome, with the two EBS cores being separated by four nucleotides. This report shows that the EBS palindrome of the human p53 promoter has a high affinity for ETS1 and ETS2 and that such **binding** interaction intracellularly is able to activate the **transcription** of a CAT reporter gene by 5-10-fold using COS cells. To investigate whether the spacing between the two EBS cores influences the DNA **binding** activity, we synthesized **oligonucleotides** with increasing distances (4,12,16, and 20 bases respectively) between the two EBS cores of the palindrome. We observed an inverse correlation between an increasing distance in the two EBS cores of the palindrome and the ETS1 and ETS2 DNA **binding** activity respectively. Interestingly, **optimal** DNA **binding** activity was observed when the distance between the two EBS cores was four bases, identical to that which occurs in the natural promoter. Furthermore we show that the p53 mRNA is expressed at higher levels in NIH3T3 cells overexpressing ETS2 gene product, suggesting that the ETS2 **transcription factor** is a likely candidate for regulating the expression of p53 in vivo.

6/3,AB/9 (Item 9 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08874639 96228295 PMID: 8642278

Importance of low affinity Elf-1 sites in the regulation of lymphoid-specific inducible gene expression.

John S; Marais R; Child R; Light Y; Leonard W J

Laboratory of Molecular Immunology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892, USA.

Journal of experimental medicine (UNITED STATES) Mar 1 1996, 183

(3) p743-50, ISSN 0022-1007 Journal Code: 2985109R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Elf-1 is an Ets family **transcription factor** that regulates a number of inducible lymphoid-specific genes, including those encoding interleukin 3 (IL-3), granulocyte/macrophage colony-stimulating factor (GM-CSF), and the IL-2 receptor (IL-2R) alpha chain. A minimal **oligonucleotide** spanning the IL-2R alpha Elf-1 site (-97/-84) bound Elf-1 poorly, but **binding** activity markedly increased when this **oligonucleotide** was multimerized or flanking sequences were added. This result is consistent with the requirement of accessory proteins for efficient Elf-1 **binding**, as has been demonstrated for the GM-CSF and

IL-3 promoters. A **binding site** selection analysis revealed the **optimal** Elf-1 consensus motif to be A(A/t)(C/a)CCGGAAGT(A/S), which is similar to the consensus motif for the related Drosophila E74 protein. This minimal high affinity site could **bind** Elf-1 and functioned as a stronger **transcription** element than the -97/-84 IL-2R alpha **oligonucleotide** when cloned upstream of a heterologous promoter. In contrast, in the context of the IL-2R alpha promoter, conversion of the naturally occurring low affinity Elf-1 site to an **optimal** site decreased inducible activation of a reporter construct in Jurkat cells. This finding may be explained by the observation that another Ets family protein, ER GB/Fli-1, can efficiently **bind** only to the **optimal** site, and in this context, interferes with Elf-1 **binding**. Therefore, high affinity Elf-1 sites may lack sufficient **binding** specificity, whereas naturally occurring low affinity sites presumably favor the association of Elf-1 in the context of accessory proteins. These findings offer an explanation for the lack of **optimal** sites in any of the known Elf-1-regulated genes.

6/3,AB/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08851683 96189074 PMID: 8628259

Binding of the Ets **factor** GA-**binding** protein to an upstream site in the **factor** IX promoter is a critical event in transactivation.

Boccia L M; Lillicrap D; Newcombe K; Mueller C R
Department of Pathology, Queen's University, Kingston, Ontario, Canada.
Molecular and cellular biology (UNITED STATES) May 1996, 16 (5)
p1929-35, ISSN 0270-7306 Journal Code: 8109087

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Factor IX is an essential vitamin K-dependent serine protease that participates in the intrinsic pathway of coagulation. The protein is expressed exclusively in the liver. The rare Leyden form of hemophilia B (inherited **factor** IX deficiency) results from point mutations in three proximal promoter elements that decrease **factor** IX expression. Recovery of expression occurs following puberty, with **factor** IX protein levels rising into the normal range. We have previously implicated the PAR domain D-site-**binding** protein (DBP) as well as an upstream element, site 5, as playing important roles in the phenotypic recovery of hemophilia B Leyden. Here we demonstrate that site 5 **binds** both the CCAAT/enhancer-**binding** protein (C/EBPalpha) and the ubiquitous Ets **factor** GA-**binding** protein (GABPalpha/beta). Transactivation of the **factor** IX promoter by the PAR proteins DBP and hepatic leukemia **factor** (HLF) is dependent on the **binding** of GABPalpha/beta to site 5, and coexpression of these two **factors** is required for **optimal** activation of this promoter. The **binding** of C/EBPalpha to site 5 also augments the activity of GABPalpha/beta. Analysis of the developmental regulation of site 5-**binding** proteins in rat liver has shown that C/EBPalpha and the GABPbeta subunit increase markedly in the 2 weeks after birth. These observations establish a functional association between the Ets **factor** GABPalpha/beta and C/EBPalpha and indicate that the two PAR proteins, DBP and HLF, may play complementary roles in **factor** IX activation. Given the developmental changes exhibited by these proteins, it is likely that they play a role in regulation of the normal **factor** IX promoter as well as promoters carrying hemophilia B Leyden mutations.

6/3,AB/11 (Item 11 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08818833 96165541 PMID: 8577737

Multiplex selection technique (MuST): an approach to clone **transcription factor binding sites**.

Nallur G N; Prakash K; Weissman S M

Department of Genetics, Boyer Center for Molecular Medicine, Yale University School of Medicine, New Haven, CT 06511, USA.

Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Feb 6 1996, 93 (3) p1184-9, ISSN 0027-8424 Journal Code: 7505876

Contract/Grant No.: CA 42556; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

We have used a multiplex selection approach to construct a library of DNA-protein interaction sites recognized by many of the DNA-binding proteins present in a cell type. An estimated minimum of two-thirds of the **binding sites** present in a library prepared from activated Jurkat T cells represent authentic **transcription factor binding sites**. We used the library for isolation of "optimal" **binding site** probes that facilitated cloning of a **factor** and to identify **binding** activities induced within 2 hr of activation of Jurkat cells. Since a large fraction of the **oligonucleotides** obtained appear to represent "optimal" **binding sites** for sequence-specific DNA-binding proteins, it is feasible to construct a catalog of consensus **binding sites** for DNA-binding proteins in a given cell type. Qualitative and quantitative comparisons of the catalogs of **binding site** sequences from various cell types could provide valuable insights into the process of differentiation acting at the level of transcriptional control.

6/3,AB/12 (Item 12 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08658598 96007455 PMID: 7559475

Determination of the consensus **binding site** for MEF2 expressed in muscle and brain reveals tissue-specific sequence constraints.

Andres V; Cervera M; Mahdavi V

Department of Cardiology, Children's Hospital and Harvard Medical School, Boston, Massachusetts 02115, USA.

Journal of biological chemistry (UNITED STATES) Oct 6 1995, 270 (40) p23246-9, ISSN 0021-9258 Journal Code: 2985121R

Contract/Grant No.: ROIHL35576; HL; NHLBI; ROIHL45425; HL; NHLBI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The myocyte-specific enhancer **factor -2** (MEF2) proteins are expressed in the three major types of muscle (skeletal, cardiac, and smooth) and function as transcriptional activators of muscle-specific and growth **factor**-regulated genes through **binding** to a canonical A/T-rich cis-element. Although MEF2 proteins are also expressed in brain, MEF2-regulated muscle-specific gene products are not detected in this tissue. To gain insight into the regulation of MEF2 function in vivo, we have selected its **optimal** DNA targets from a library of degenerate **oligonucleotides** using anti-MEF2A antibodies and cell extracts from skeletal muscle, heart, and brain. The consensus **binding site** in these three tissues contains an indistinguishable core motif, 5'-CT(A/t)(a/t)AAATAG-3'. However, the **optimal** target for MEF2 expressed in the brain shows additional sequence constraints (5'-TGTTACT(A/t)(a/t)AAATAGA(A/t)-3') that are not observed in the

sequences selected with skeletal and cardiac muscle extracts. Thus, differences in DNA **binding** preferences of MEF2 proteins in muscle and brain may contribute to tissue-specific gene expression during myogenesis and neurogenesis.

6/3,AB/13 (Item 13 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08594132 95352207 PMID: 7626222

Identification of **optimized** target sequences for the GLI3 zinc finger protein.

Vortkamp A; Gessler M; Grzeschik K H
Institut fur Humangenetik, Marburg, Germany.

DNA and cell biology (UNITED STATES) Jul 1995, 14 (7) p629-34,
ISSN 1044-5498 Journal Code: 9004522

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

GLI3 represents an important control gene for development and differentiation of several body structures. Reduction in gene dosage already leads to severe perturbation, especially of limb morphogenesis. The gene encodes a zinc finger protein that likely functions as a transcriptional modulator. Because the five zinc fingers should be capable of recognizing an extended stretch of genomic DNA, we sought to identify sequences bound by GLI3 that may facilitate the search for target genes acting downstream of GLI3. Starting from the nonamer DNA **binding** sequence of the highly related GLI protein, we employed an **oligonucleotide** selection protocol to determine an **optimized binding** sequence for the GLI3 protein. The resulting sequence bound by the GLI3 zinc fingers consists of 16 nucleotides and shows a high degree of similarity to sequences bound by the GLI and tra-1 proteins. Comparison with protein-DNA interactions in the known crystal structure of the GLI-DNA complex suggests relevant interactions of additional amino acids of GLI3 with its target site. The newly identified GLI3 target sequence should prove very useful for both the structural analysis of the protein-DNA complex and the search for genes whose expression is subject to regulation by the GLI3 gene product.

6/3,AB/14 (Item 14 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08535373 95293230 PMID: 7774815

A STAT protein domain that determines DNA sequence recognition suggests a novel DNA-**binding** domain.

Horvath C M; Wen Z; Darnell J E

Laboratory of Molecular Cell Biology, Rockefeller University, New York, New York 10021, USA.

Genes & development (UNITED STATES) Apr 15 1995, 9 (8) p984-94

, ISSN 0890-9369 Journal Code: 8711660

Contract/Grant No.: AI32489; AI; NIAID; AI34420; AI; NIAID; NS09230; NS; NINDS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Stat1 and Stat3 are two members of the ligand-activated **transcription factor** family that serve the dual functions of signal transducers and activators of **transcription**. Whereas the two proteins select very similar (not identical) **optimum binding sites** from random **oligonucleotides**, differences in their **binding** affinity were readily apparent with natural STAT-

binding sites. To take advantage of these different affinities, chimeric Stat1:Stat3 molecules were used to locate the amino acids that could discriminate a general **binding site** from a specific **binding site**. The amino acids between residues approximately 400 and approximately 500 of these approximately 750-amino-acid-long proteins determine the DNA-**binding site** specificity. Mutations within this region result in Stat proteins that are activated normally by tyrosine phosphorylation and that dimerize but have greatly reduced DNA-**binding** affinities.

6/3,AB/15 (Item 15 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08522901 95298342 PMID: 7779405

Triplex-forming **oligonucleotide binding** represses
transcription of the human c-erbB gene in glioma.

Okada T; Yamaguchi K; Yamashita Y
Department of Neurosurgery, Kanazawa University School of Medicine,
Japan.

Growth factors (Chur, Switzerland) (SWITZERLAND) 1994, 11 (4)
p259-70, ISSN 0897-7194 Journal Code: 9000468

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Mixed purine-pyrimidine oligodeoxynucleotides were designed to form collinear DNA triplexes with pyrimidine-rich elements in the EGFR gene promoter. Their effects as mediators of human epidermal growth factor receptor (EGFR) gene **transcription** and subsequent gene expression were evaluated using human squamous cell carcinoma (A431) and human glioma cell line (U251MG and U87MG). Gel shift analysis indicated that the **oligonucleotide** forms a collinear triplex within the duplex Sp-1 **binding site**. An in vitro assay system revealed a correlation between triplex formation and the repression of EGFR **transcription**. We postulate that guanine residues are not always **optimum** in apposition to G-C pairs to form triple helices in the target. Site-specific oligodeoxynucleotides **binding** to a DNA duplex may serve as the basis for an alternative program of gene control in vitro.

6/3,AB/16 (Item 16 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08322508 95080131 PMID: 7988448

Identification of deoxyribonucleic acid sequences that **bind**
retinoid-X receptor-gamma with high affinity.

Dowhan D H; Downes M; Sturm R A; Muscat G E
University of Queensland, Center for Molecular Biology and Biotechnology,
Ritchie Research Laboratories, St. Lucia, Australia.

Endocrinology (UNITED STATES) Dec 1994, 135 (6) p2595-607,
ISSN 0013-7227 Journal Code: 0375040

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The retinoid-X receptor (RXR) family (-alpha, -beta and -gamma) forms homodimers that **bind** to a number of retinoid-X response elements and trans-activate gene expression in a retinoid-dependent manner. Although, the RXRs are known to **bind** tandem direct repeats (DR) of the hexamer, RGGTCA, separated by 1 nucleotide, it is not known whether these represent the **optimal** and/or only recognition sequences. We, therefore, used a nonbiased strategy to identify sequences that efficiently bound RXR gamma, an isoform preferentially expressed in cardiac and skeletal muscle tissue.

We performed **binding site** selection with bacterially expressed RXR gamma bound to glutathione-agarose and a pool of random sequences to derive a consensus DNA-**binding site** for RXR gamma. We analyzed a total of 41 individually selected **oligonucleotides** and found that RXR gamma bound with high affinity to motifs that were accommodated by the consensus AAGRNCAAAGGTCAA/cR. We observed that the majority of the sequences that formed complexes with RXR gamma in electrophoretic mobility shift analysis were DR-1 motifs; however, DR- motifs separated by 2, 4, and 8 nucleotides and a palindrome-0 motif were also demonstrated to interact with RXR gamma. Mutagenesis of the derived sequences indicated that both RGGTCA motifs were required for high affinity **binding** to RXR gamma. These derived sequences conferred appropriate 9-cis- and all-trans-retinoic acid (RA) responses to a thymidine kinase promoter. Furthermore, supershift experiments with a RXR antibody verified that these sequences specifically interacted with RXR in nuclear extracts derived from C2C12 muscle cells. In conclusion, this study rigorously defines the range of DR motifs that can recognize RXR and regulate gene expression in a RA-dependent fashion. The derived consensus accommodates retinoid-X response elements that have been identified in a diverse range of genes trans-activated by 9-cis-RA via the RXR family.

6/3,AB/17 (Item 17 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08263433 95021189 PMID: 7523856

A novel hepatocytic **transcription factor** that **binds** the alpha-fetoprotein promoter-linked coupling element.

Wen P; Locker J

Department of Pathology, University of Pittsburgh, Pennsylvania 15261.

Molecular and cellular biology (UNITED STATES) Oct 1994, 14

(10) p6616-26, ISSN 0270-7306 Journal Code: 8109087

Contract/Grant No.: CA43909; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

We recently characterized a promoter-linked coupling element (PCE) in the rat alpha-fetoprotein (AFP) gene required for strong transcriptional stimulation by distant enhancers (P. Wen, N. Crawford, and J. Locker, Nucleic Acids Res. 21:1911-1918, 1993). In this study, **oligonucleotide** gel retardation and competition experiments defined the PCE as a 12-bp **binding site**, TGTCTTGAACA, an imperfect inverted repeat from -166 to -155 near the AFP promoter. A **factor** that bound this site (PCF) was abundant in HepG2 nuclear extracts and detectable in extracts from several other AFP-producing hepatocarcinoma cell lines and fetal liver. Hepatocytic cell lines that did not express AFP, nonhepatocytic cell lines, adult liver, and fetal brain did not show the **factor**. Experiments excluded the possibility that PCF activity was due to **binding** of glucocorticoid receptor or an AP1-like **factor** that bound overlapping sites. Competition experiments with several mutant **oligonucleotides** determined that the **optimum** PCF **binding site** was TGTCTTGAAC(A/T). Mutations decreased **binding** or totally abolished **binding** activity. In expression plasmids, PCE mutations strongly reduced gene expression. UV cross-linking to a PCE probe identified peptide bands near 34 kDa. PCF was purified by heparin-Sepharose chromatography followed by affinity **binding** to oligomerized PCE DNA. The product resolved as a complex of three peptides (PCF alpha 1, PCF alpha 2, and PCF beta, 32 to 34 kDa) on sodium dodecyl sulfate-acrylamide gels. The peptide sizes and gel patterns are unlike those of any of the well-described hepatic **transcription factors**, and the **binding site** has not been previously reported. PCF thus appears to be a novel **transcription factor**.

6/3,AB/18 (Item 18 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08195665 94329439 PMID: 8052536

Base preferences for DNA **binding** by the bHLH-Zip protein USF: effects of MgCl₂ on specificity and comparison with **binding** of Myc family members.

Bendall A J; Molloy P L

CSIRO Division of Biomolecular Engineering, North Ryde, NSW, Australia.

Nucleic acids research (ENGLAND) Jul 25 1994, 22 (14) p2801-10

, ISSN 0305-1048 Journal Code: 0411011

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Studies of the DNA **binding** specificity of **transcription factors** belonging to the basic helix-loop-helix (bHLH) family have identified the so-called E-box, CACGTG, as being a high affinity specific **binding** sequence for this class of DNA **binding** proteins. **Binding** sequences for HeLa USF were selected from an initially random population of 20 bp sequences, defining the **optimum** USF **binding** sequence as R-5Y-4C-3A-2C-1G+1T+2G+3R+4Y+5. The significance of the flanking bases was further demonstrated by showing that USF and the related proteins c-Myc and Max discriminate between CACGTG-type E-boxes and that the primary means of discrimination appears to be the identity of the nucleotide at +/- 4, the presence of a T at -4 being inhibitory to **binding** by Myc but not by USF or Max. This suggests one mechanism by which bHLH **factors** are partitioned between multiple potential **binding** sequences in the promoters and enhancers of viral and cellular genes. It was also demonstrated that MgCl₂ has a significant influence on USF DNA **binding** specificity. A broader range of USF **binding sites** was selected in the absence of MgCl₂, conforming to the altered half-site consensus GTGaY. **Binding** studies with specific **oligonucleotides** demonstrated significantly improved tolerance to sequence variation at positions 1, 4, and to a lesser extent 5, of the GTGRY consensus in the absence of MgCl₂. The results indicate that Mg²⁺ ions have an integral role in the formation of the USF-DNA complex.

6/3,AB/19 (Item 19 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08180596 94316511 PMID: 8041627

The RNA polymerase I **transcription factor** UBF is a sequence-tolerant HMG-box protein that can recognize structured nucleic acids.

Copenhaver G P; Putnam C D; Denton M L; Pikaard C S

Biology Department, Washington University, St Louis, MO 63130.

Nucleic acids research (ENGLAND) Jul 11 1994, 22 (13) p2651-7,

ISSN 0305-1048 Journal Code: 0411011

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Upstream **Binding Factor** (UBF) is important for activation of ribosomal RNA **transcription** and belongs to a family of proteins containing nucleic acid **binding** domains, termed HMG-boxes, with similarity to High Mobility Group (HMG) chromosomal proteins. Proteins in this family can be sequence-specific or highly sequence-tolerant **binding** proteins. We show that Xenopus UBF can be classified among the sequence-tolerant class. Methylation interference assays using enhancer DNA probes failed to reveal any critical nucleotides required for UBF

binding. Selection by UBF of **optimal binding sites** among a population of enhancer **oligonucleotides** with randomized sequences also failed to reveal any consensus sequence. The minor groove specific drugs chromomycin A3, distamycin A and actinomycin D competed against UBF for enhancer **binding**, suggesting that UBF, like other HMG-box proteins, probably interacts with the minor groove. UBF also shares with other HMG box proteins the ability to **bind** synthetic cruciform DNA. However, UBF appears different from other HMG-box proteins in that it can **bind** both RNA (tRNA) and DNA. The sequence-tolerant nature of UBF-nucleic acid interactions may accommodate the rapid evolution of ribosomal RNA gene sequences.

6/3,AB/20 (Item 20 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08161148 94297930 PMID: 8025823

A nuclear **factor** that **binds** to a dyad-symmetric sequence with a CGTCA motif in the 5'-upstream region of the sweet potato beta-amylase gene.

Ishiguro S; Tanaka M; Kojimoto A; Kato M; Iwabuchi M; Nakamura K
Laboratory of Biochemistry, School of Agriculture, Nagoya University, Japan.

Plant & cell physiology (JAPAN) Jun 1993, 34 (4) p567-76,
ISSN 0032-0781 Journal Code: 9430925

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

A nuclear extract from petioles of sweet potato protected several sites in the 5'-upstream region of a gene for beta-amylase from DNase I digestion. One of these sites, located at a region around 800-base pairs upstream from the **transcription** start site, having an imperfect palindromic sequence of CGTCACGTCACG, was designated the R-box. The site contained tandemly duplicated CGTCA sequences, referred to below as 5'- and 3'-CGTCA. Competition experiments in gel mobility shift assays with mutant R-box **oligonucleotides** indicated that mutations in bases outside the 3'-CGTCA of the R-box do not severely affect the **binding**. By contrast, single-base substitutions in any one base of the 3'-CGTCA greatly abolished the **binding** even when the mutated R-boxes contained intact 5'-CGTCA. However, **oligonucleotides** with mutations in the 3'-CGTCA had the ability to **bind** the nuclear **factor** when additional mutations were introduced to create a partially palindromic sequence containing the CGTCA sequence in its 3'-half on the opposite strand. These results indicate that the CGTCA sequence alone is not sufficient for the **binding** of the R-box **binding factor** (RBF) and that the RBF **binds** to the sequence with partial dyad symmetry that contains the CGTCA motif in its 3'-half. The **optimum** sequence for the **binding** of the RBF is suggested to be a palindromic octameric sequence TGACGTCA, which is identical to the consensus sequence of the cAMP-responsive element (CRE) of animal genes. Bacterially produced HBP-1b of wheat bound to the R-box, and its **binding** to mutated R-boxes was similar to that of RBF, suggesting that the RBF belongs to a family of bZIP-type plant nuclear **factors** that **bind** to CGTCA-related sequences. However, several differences between the RBF and HBP-1b were also noted.

6/3,AB/21 (Item 21 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08009413 94146013 PMID: 8312279

Regulatory mechanism of human **factor IX** gene: protein **binding** at the Leyden-specific region.

Kurachi S; Furukawa M; Salier J P; Wu C T; Wilson E J; French F S;
Kurachi K

Department of Human Genetics, University of Michigan Medical Center, Ann Arbor 48109-0618.

Biochemistry (UNITED STATES) Feb 15 1994, 33 (6) p1580-91,
ISSN 0006-2960 Journal Code: 0370623

Contract/Grant No.: HL 38644; HL; NHLBI

Erratum in Biochemistry 1995 Oct 31;34(43) 14270

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Hemophilia B-Leyden is characterized by the gradual amelioration of bleeding after the onset of puberty. All Leyden phenotype mutations found to date lie within the Leyden-specific region, which spans roughly nt-40 to +20 in the 5' end of the human **factor IX** gene. With HepG2 cell nuclear extracts, the Leyden-specific region and its immediate neighboring region of the normal **factor IX** gene showed five DNase I footprints: FP-I (nt +4 to +19), FP-II (nt -16 to -3), FP-III (nt -27 to -19), FP-IV (nt -67 to -49), and FP-V (nt -99 to -77). Protein **binding** affinities of short **oligonucleotides** containing sequences of FP-I, FP-II, or FP-III were substantially reduced in the presence of Leyden phenotype mutations in these areas, correlating well with the negative effects of these mutations on **factor IX** gene expression. A Leyden phenotype mutation at nt -20 (T to A) caused a loss of both footprints FP-III and FP-II but generated a new footprint, FP-III' (nt -34 to -23), partially overlapping with FP-III, indicating mutation-dependent competitive protein **binding** at these sites. Although the FP-III' area contains an androgen responsive element-like sequence, the nuclear protein that **binds** at FP-III' is not androgen receptor. The protein was not recognized by anti-androgen receptor antibody and, furthermore, was present not only in liver but also in both androgen receptor-positive and androgen receptor-negative cells in electrophoretic mobility shift assays. The nuclear concentration of this protein increased significantly upon treatment of the HepG2 cells with testosterone. Its **binding** affinity to an **oligonucleotide** (-32sub) containing the FP-III' sequence was greatly reduced in the presence of exogenous androgen receptor, suggesting a possible interaction of this protein with androgen receptor. The affinities of both this protein and a protein which **binds** to FP-III (presumably HNF-4) to -32sub with a mutation at nt -26 were grossly lowered. These findings suggest that the amelioration of hemophilia B-Leyden with a mutation at nt -20 after puberty involves **binding** of a specific non-androgen receptor nuclear protein at FP-III' and it is able to substitute for the function of a protein bound at FP-III in the normal gene **optimally** through its elevated interaction with androgen receptor upon a surge of testosterone. (ABSTRACT TRUNCATED AT 400 WORDS)

6/3,AB/22 (Item 22 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07745519 93268288 PMID: 8497258

Purification of early-B-cell **factor** and characterization of its DNA-**binding** specificity.

Travis A; Hagman J; Hwang L; Grosschedl R

Howard Hughes Medical Institute, Department of Microbiology, University of California, San Francisco 94143-0414.

Molecular and cellular biology (UNITED STATES) Jun 1993, 13 (6)

p3392-400, ISSN 0270-7306 Journal Code: 8109087

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Early-B-cell **factor** (EBF) is a nuclear protein that recognizes a

functionally important sequence in the promoter of the mb-1 gene. Like the mb-1 gene, which encodes an immunoglobulin-associated protein, EBF is specifically expressed in the early stages of B-lymphocyte differentiation. We purified EBF by sequence-specific DNA affinity chromatography and examined its biochemical properties and DNA-binding specificity. Crude nuclear extract and affinity-purified EBF generated protein-DNA complexes with the mb-1 promoter that were indistinguishable in electrophoretic mobility shift and DNase I footprint assays. Fractionation of affinity-purified EBF by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and renaturation of isolated polypeptides indicated that EBF DNA-binding activity could be reconstituted from polypeptides with molecular masses of 62 to 65 kDa. Gel filtration chromatography suggested that native EBF has a molecular mass of 140 kDa, if a globular shape of the protein is assumed. Thus, EBF appears to be a dimer with subunits of 62 to 65 kDa. To characterize the DNA-binding specificity of purified EBF, we performed two sets of experiments. First, we examined various mutant EBF-binding sites for interaction with purified EBF in an electrophoretic mobility shift assay. Second, we used oligonucleotides containing pairs of randomized bases in a binding-site selection and amplification experiments to determine a preferred sequence for DNA binding by EBF. Taken together, the results of these experiments indicated that EBF recognizes variations on the palindromic sequence 5'-ATTCCCNNGGAAT, with an optimal spacer of 2 bp between the half-sites.

6/3,AB/23 (Item 23 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07678053 93204981 PMID: 8096059

Nucleotides flanking a conserved TAAT core dictate the DNA binding specificity of three murine homeodomain proteins.

Catron K M; Iler N; Abate C

Center for Advanced Biotechnology and Medicine, Rutgers University, Piscataway, New Jersey.

Molecular and cellular biology (UNITED STATES) Apr 1993, 13 (4)

p2354-65, ISSN 0270-7306 Journal Code: 8109087

Contract/Grant No.: HD29446-01; HD; NICHD

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Murine homeobox genes play a fundamental role in directing embryogenesis by controlling gene expression during development. The homeobox encodes a DNA binding domain (the homeodomain) which presumably mediates interactions of homeodomain proteins with specific DNA sites in the control regions of target genes. However, the bases for these selective DNA-protein interactions are not well defined. In this report, we have characterized the DNA binding specificities of three murine homeodomain proteins, Hox 7.1, Hox 1.5, and En-1. We have identified optimal DNA binding sites for each of these proteins by using a random oligonucleotide selection strategy. Comparison of the sequences of the selected binding sites predicted a common consensus site that contained the motif (C/G)TAATTG. The TAAT core was essential for DNA binding activity, and the nucleotides flanking this core directed binding specificity. Whereas variations in the nucleotides flanking the 5' side of the TAAT core produced modest alterations in binding activity for all three proteins, perturbations of the nucleotides directly 3' of the core distinguished the binding specificity of Hox 1.5 from those of Hox 7.1 and En-1. These differences in binding activity reflected differences in the dissociation rates rather than the equilibrium constants of the protein-DNA complexes. Differences in DNA binding specificities observed in vitro may contribute to selective interactions of homeodomain proteins with potential binding sites in the

control regions of target genes.

6/3,AB/24 (Item 24 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07587388 93100825 PMID: 8380087

A mouse mammary tumor virus promoter element near the **transcription** initiation site.

Pierce J; Fee B E; Toohey M G; Peterson D O

Department of Biochemistry and Biophysics, Texas A&M University, College Station 77843-2128.

Journal of virology (UNITED STATES) Jan 1993, 67 (1) p415-24,

ISSN 0022-538X Journal Code: 0113724

Contract/Grant No.: CA32695; CA; NCI; CA48041; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Transcription from the promoter of mouse mammary tumor virus is subject to both positive and negative control by cellular **factors**, and proviral promoter elements that mediate a basal level of **transcription** must in some way respond to these cellular regulatory signals. Several such elements, including a TATA box, a region containing three octamer-related sequences, and a **binding site** for nuclear **factor 1**, have been previously defined. Additional promoter mutations have allowed a fourth basal promoter element to be identified near the **transcription** initiation site between +2 and +10. Sequence alterations within this element affect **transcription** both in vivo and in vitro. Gel electrophoresis mobility shift and DNase I footprinting assays define a nuclear protein, termed initiation site-**binding** protein, that specifically recognizes this region of the promoter. **Optimal** levels of **transcription** from the mouse mammary tumor virus promoter require initiation site-**binding** protein, as demonstrated by a correlation between protein affinity and transcriptional activity and by specific inhibition of **transcription** in vitro by an **oligonucleotide** capable of titrating the protein from transcriptionally active fractions.

6/3,AB/25 (Item 25 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07495631 93024383 PMID: 1406630

Selection of **optimal** kappa B/Rel DNA-**binding** motifs: interaction of both subunits of NF-kappa B with DNA is required for transcriptional activation.

Kunsch C; Ruben S M; Rosen C A

Department of Gene Regulation, Roche Institute of Molecular Biology, Nutley, New Jersey 07110.

Molecular and cellular biology (UNITED STATES) Oct 1992, 12

(10) p4412-21, ISSN 0270-7306 Journal Code: 8109087

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Analysis of the p50 and p65 subunits of the NF-kappa B **transcription factor** complex has revealed that both proteins can interact with related DNA sequences through either homo- or heterodimer formation. In addition, the product of the proto-oncogene c-rel can **bind** to similar DNA motifs by itself or as a heterodimer with p50 or p65. However, these studies have used a limited number of known kappa B DNA motifs, and the question of the **optimal** DNA sequences preferred by each homodimer has not been addressed. Using purified recombinant p50, p65,

and c-Rel proteins, **optimal DNA-binding motifs** were selected from a pool of random **oligonucleotides**. Alignment of the selected sequences allowed us to predict a consensus sequence for **binding** of the individual homodimeric Rel-related proteins, and DNA-protein **binding** analysis of the selected DNA sequences revealed sequence specificity of the proteins. Contrary to previous assumptions, we observed that p65 homodimers can interact with a subset of DNA sequences not recognized by p50 homodimers. Differential **binding** affinities were also obtained with p50- and c-Rel-selected sequences. Using either a p50- or p65-selected kappa B motif, which displayed differential **binding** with respect to the other protein, little to no **binding** was observed with the heterodimeric NF-kappa B complex. Similarly, in transfection experiments in which the selective kappa B **binding sites** were used to drive the expression of a chloramphenicol acetyltransferase reporter construct, the p65- and p50-selected motifs were activated only in the presence of p65 and p50/65 (a chimeric protein with the p50 DNA **binding** domain and p65 activation domain) expression vectors, respectively, and neither demonstrated a significant response to stimuli that induce NF-kappa B activity. These findings demonstrate that interaction of both subunits of the heterodimeric NF-kappa B complex with DNA is required for DNA **binding** and transcriptional activation and suggest that transcriptional activation mediated by the individual rel-related proteins will differ dramatically, depending on the specific kappa B motifs present.

6/3,AB/26 (Item 26 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07296336 92249179 PMID: 1811948

A G-string positive cis-regulatory element in the LpS1 promoter **binds** two distinct nuclear **factors** distributed non-uniformly in *Lytechinus pictus* embryos.

Xiang M; Lu S Y; Musso M; Karsenty G; Klein W H

Department of Biochemistry and Molecular Biology, University of Texas MD Anderson Cancer Center, Houston 77030.

Development (Cambridge, England) (ENGLAND) Dec 1991, 113 (4)

p1345-55, ISSN 0950-1991 Journal Code: 8701744

Contract/Grant No.: HD22619; HD; NICHD

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The LpS1 alpha and beta genes of *Lytechinus pictus* are activated at the late cleavage stage of embryogenesis, with LpS1 mRNAs accumulating only in lineages contributing to aboral ectoderm. We had shown previously that 762 bp of 5' flanking DNA from the LpS1 beta gene was sufficient for proper temporal and aboral ectoderm specific expression. In the present study, we identified a strong positive cis-regulatory element at -70 bp to -75 bp in the LpS1 beta promoter with the sequence (G)6 and a similar, more distal cis-element at -721 bp to -726 bp. The proximal 'G-string' element interacted with two nuclear **factors**, one specific to ectoderm and one to endoderm/mesoderm nuclear extracts, whereas the distal G-string element interacted only with the ectoderm **factor**. The ectoderm and endoderm/mesoderm G-string **factors** were distinct based on their migratory behavior in electrophoretic mobility shift assays, **binding site** specificities, salt **optima** and EDTA sensitivity. The proximal G-string element shared homology with a **binding site** for the mammalian **transcription factor** IF1, a protein that **binds** to negative cis-regulatory elements in the mouse alpha 1(I) and alpha 2(I) collagen gene promoters. Competition experiments using wild-type and mutant **oligonucleotides** indicated that the ectoderm G-string **factor** and IF1 have similar recognition sites. Partially purified IF1 specifically bound to an **oligonucleotide** containing the proximal

G-string of LpS1 beta. From our results, we suggest that the ectoderm G-string **factor**, a member of the G-rich DNA-binding protein family, activates the LpS1 gene in aboral ectoderm cells by **binding** to the LpS1 promoter at the proximal G-string site.

6/3,AB/27 (Item 27 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07178668 92115325 PMID: 1766672

High-affinity DNA-protein interactions of the cellular ETS1 protein: the determination of the ETS **binding** motif.

Fisher R J; Mavrothalassitis G; Kondoh A; Papas T S
Laboratory of Cellular Biochemistry, Program Resources, Inc./DynCorp,
Frederick, Maryland 21702-1012.

Oncogene (ENGLAND) Dec 1991, 6 (12) p2249-54, ISSN 0950-9232
Journal Code: 8711562

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

ETS1 protein purified from CEM cells was used to select its **optimum** DNA-binding sequence (pu) G/CCaGGA-AGTc (py). The sequence CCGGAAGT (ETS1-3) was preferred 5:1 over CAGGAAGT (PEA3). Quantitative electrophoretic mobility-shift assays (EMSA) indicated that the purified ETS1 protein **binds** to either ETS1-3 or PEA3 **oligonucleotide** probes with high affinity ($K_a = 0.5-4.0 \times 10^{10} \text{ M}^{-1}$) and that the purified ETS1 has different **binding** capacities for ETS1-3 and PEA3 **oligonucleotide** probes. The ETS1 protein **binds** 2-5 times more ETS1-3 than PEA3. Competitive **binding** experiments showed that the ETS1-3 and PEA3 probes effectively compete for the **binding** of ETS1-3. However, changing the core DNA-binding sequence from GGAA to AGAA eliminates competition. Since the human ETS1 protein selected the same DNA sequence from a mixture of random **oligonucleotides** as did the Drosophila E74A protein (one of the most divergent members of the ETS family), this strongly suggests that all proteins containing the ETS 85 amino acid domain (sequences which define the ETS family) will **bind** to the same sequence.

6/3,AB/28 (Item 28 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

06949945 91260710 PMID: 1675428

DNA-binding specificity of the fushi tarazu homeodomain.

Florence B; Handrow R; Laughon A

Departments of Genetics, University of Wisconsin, Madison 53706.

Molecular and cellular biology (UNITED STATES) Jul 1991, 11 (7)

p3613-23, ISSN 0270-7306 Journal Code: 8109087

Contract/Grant No.: HD23820; HD; NICHD

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The fushi tarazu (ftz) gene of Drosophila melanogaster encodes a homeodomain-containing **transcription factor** that functions in the formation of body segments. Here we report an analysis of the DNA-binding properties of the ftz homeodomain in vitro. We provide evidence that the homeodomain **binds** to DNA as a monomer, with an equilibrium dissociation constant of $2.5 \times 10^{-11} \text{ M}$ for **binding** to a consensus **binding site**. A single ftz **binding site** occupies 10 to 12 bp, as judged by the ability of protein bound at one site to interfere with **binding** to an adjacent site. These experiments also demonstrated a lack of cooperative **binding** between ftz homeodomains.

Analysis of single-nucleotide substitutions over an 11-bp sequence shows that a stretch of 6 bp is critical for **binding**, with an **optimal** sequence of 5'CTAATTA3'. These data correlate well with recent structural evidence for base-specific contact at these positions. In addition, we found that sequences flanking the region of direct contact have effects on DNA **binding** that could be of biological significance.

6/3,AB/29 (Item 29 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

06806758 91117251 PMID: 1990287
trans-Activation of a globin promoter in nonerythroid cells.
Evans T; Felsenfeld G
Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland 20892.
Molecular and cellular biology (UNITED STATES) Feb 1991, 11 (2)
p843-53, ISSN 0270-7306 Journal Code: 8109087
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

We show that expression in fibroblasts of a single cDNA, encoding the erythroid DNA-**binding** protein Eryf1 (GF-1, NF-E1), very efficiently activates **transcription** of a chicken alpha-globin promoter, trans-Activation in these cells occurred when Eryf1 bound to a single site within a minimal globin promoter. In contrast, efficient activation in erythroid cells required multiple Eryf1 **binding sites**. Our results indicate that mechanisms exist that are capable of modulating the trans-acting capabilities of Eryf1 in a cell-specific manner, without affecting DNA **binding**. The response of the minimal globin promoter to Eryf1 in fibroblasts was at least as great as for **optimal** constructions in erythroid cells. Therefore, the assay provides a very simple and sensitive system with which to study gene activation by a tissue-specific **factor**.

6/3,AB/30 (Item 30 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

06803595 91105861 PMID: 1846322
An inhibitory domain of E12 **transcription factor** prevents DNA **binding** in E12 homodimers but not in E12 heterodimers.
Sun X H; Baltimore D
Whitehead Institute for Biomedical Research, Cambridge, Massachusetts 02142.
Cell (UNITED STATES) Jan 25 1991, 64 (2) p459-70, ISSN 0092-8674 Journal Code: 0413066
Contract/Grant No.: GM39458; GM; NIGMS
Erratum in Cell 1991 Aug 9;66(3) 423
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The kappa E2 sequence **binding** proteins, E12 and E47, are generated by alternative splicing of the E2A gene, giving closely related basic and helix-loop-helix structures crucial for DNA **binding** and dimerization. Measurements of dimerization constants and **binding** strengths to the **optimal** DNA sequence (the kappa E2 site or its near relatives) showed that E47 homodimers and MyoD heterodimers with E12 or E47 dimerized and bound avidly, but E12 homodimerized efficiently and bound to DNA poorly; MyoD homodimerized poorly and bound strongly. An inhibitory domain N-terminal to the basic region of E12 prevents E12 homodimers but not E12/MyoD heterodimers from **binding** to DNA. Thus, E47 **binds** to

DNA both as a heterodimer with MyoD and as a homodimer, while E12 and MyoD bind to DNA efficiently only as heterodimers.

6/3,AB/31 (Item 31 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06800257 91093132 PMID: 1985897

DNA binding of purified transcription factor NF-kappa B. Affinity, specificity, Zn²⁺ dependence, and differential half-site recognition.

Zabel U; Schreck R; Baeuerle P A
Laboratory for Molecular Biology, Ludwig-Maximilians-University Munich, Martinsried, Federal Republic of Germany.

Journal of biological chemistry (UNITED STATES) Jan 5 1991, 266

(1) p252-60, ISSN 0021-9258 Journal Code: 2985121R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

A rapid purification procedure for the NF-kappa B transcription factor from the cytosol of human placenta is demonstrated which exploits the insensitivity of the NF-kappa B-DNA complex toward the intercalating agent chloroquine. Purified NF-kappa B required 100 mM KCl or NaCl and a pH of 7.5 to optimally bind to DNA. Equilibrium of binding was reached within less than 5 min in the absence of competitor DNA and after 1 h in the presence of 0.1 mg/ml poly(dI-dC). DNA binding of NF-kappa B was specifically blocked by the chelating agent 1,10-orthophenanthroline and could only be reconstituted by addition of Zn²⁺. Under optimal binding conditions, the dissociation constant for the complex of the purified NF-kappa B with its most frequent cognate DNA motif 5'-GGGACTTTC-3' was in the range of 10⁻¹² to 10⁻¹³ M. Various other cis-acting kappa B motifs were recognized by NF-kappa B with lower affinities. A comparative analysis of known NF-kappa B-binding sites and competition experiments with synthetic polynucleotides and oligonucleotides encompassing only one half-site or single-stranded kappa B motifs suggested that the two DNA-binding monomers in the NF-kappa B protein complex can interact differentially with the half-sites of the decameric cognate motif.

6/3,AB/32 (Item 32 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06677730 90377197 PMID: 2204805

Mutations that define the optimal half-site for binding yeast GCN4 activator protein and identify an ATF/CREB-like repressor that recognizes similar DNA sites.

Sellers J W; Vincent A C; Struhl K
Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115.

Molecular and cellular biology (UNITED STATES) Oct 1990, 10

(10) p5077-86, ISSN 0270-7306 Journal Code: 8109087

Contract/Grant No.: GM 30186; GM; NIGMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The yeast GCN4 transcriptional activator protein binds as a dimer to a dyad-symmetric sequence, indicative of a protein-DNA complex in which two protein monomers interact with adjacent half-sites. However, the optimal GCN4 recognition site, ATGA(C/G)TCAT, is inherently asymmetric because it contains an odd number of base pairs and because mutation of the central C.G base pair strongly reduces specific DNA

binding . From this asymmetry, we suggested previously that GCN4 interacts with nonequivalent and possibly overlapping half-sites (ATGAC and ATGAG) that have different affinities. Here, we examine the nature of GCN4 half-sites by creating symmetrical derivatives of the **optimal** GCN4 **binding** sequence that delete or insert a single base pair at the center of the site. In vitro, GCN4 bound efficiently to the sequence ATGACGTCAT, whereas it failed to **bind** to ATGAGCTCAT or ATGATCAT. These observations strongly suggest that (i) GCN4 specifically recognizes the central base pair, (ii) the **optimal** half-site for GCN4 **binding** is ATGAC, not ATGAG, and (iii) GCN4 is a surprisingly flexible protein that can accommodate the insertion of a single base pair in the center of its compact **binding site**. The ATGACGTCAT sequence strongly resembles sites bound by the yeast and mammalian ATF/CREB family of proteins, suggesting that GCN4 and the ATF/CREB proteins recognize similar half-sites but have different spacing requirements. Unexpectedly, in the context of the his3 promoter, the ATGACGTCAT derivative reduced **transcription** below the basal level in a GCN4-independent manner, presumably reflecting DNA **binding** by a distinct ATF/CREB-like repressor protein. In other promoter contexts, however, the same site acted as a weak upstream activating sequence.

6/3,AB/33 (Item 33 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

06429819 90136576 PMID: 2153920

Hormonal induction of transfected genes depends on DNA topology.

Pina B; Hache R J; Arnemann J; Chalepakakis G; Slater E P; Beato M

Institut fur Molekularbiologie und Tumorforschung, Philipps Universitat, Marburg, Federal Republic of Germany.

Molecular and cellular biology (UNITED STATES) Feb 1990, 10 (2)

p625-33, ISSN 0270-7306 Journal Code: 8109087

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Plasmids containing the hormone regulatory element of mouse mammary tumor virus linked to the thymidine kinase promoter of herpes simplex virus and the reporter gene chloramphenicol acetyltransferase of Escherichia coli respond to glucocorticoids and progestins when transfected into appropriate cells. In the human mammary tumor cell line T47D, the response to progestins, but not to glucocorticoids, is highly dependent on the topology of the transfected DNA. Although negatively supercoiled plasmids respond **optimally** to the synthetic progestin R5020, their linearized counterparts exhibit markedly reduced progestin inducibility. This is not due to changes in the efficiency of DNA transfection, since the amount of DNA incorporated into the cell nucleus is not significantly dependent on the initial topology of the plasmids. In contrast, cotransfection experiments with glucocorticoid receptor cDNA in the same cell line show no significant influence of DNA topology on induction by dexamethasone. A similar result was obtained with fibroblasts that contain endogenous glucocorticoid receptors. When the distance between receptor-**binding sites** or between the **binding sites** and the promoter was increased, the dependence of progestin induction on DNA topology was more pronounced. In contrast to the original plasmid, these constructs also revealed a similar topological dependence for induction by glucocorticoids. The differential influence of DNA topology is not due to differences in the affinity of the two hormone receptors for DNA of various topologies, but probably reflects an influence of DNA topology on the interaction between different DNA-bound receptor molecules and between receptors and other **transcription factors**.

6/3,AB/34 (Item 34 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

05965773 89053051 PMID: 3192075

Cytosine methylation prevents **binding** to DNA of a HeLa cell **transcription factor** required for **optimal** expression of the adenovirus major late promoter.

Watt F; Molloy P L

CSIRO Division of Biotechnology, Laboratory for Molecular Biology, North Ryde, NSW, Australia.

Genes & development (UNITED STATES) Sep 1988, 2 (9) p1136-43,

ISSN 0890-9369 Journal Code: 8711660

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Cytosine methylation within CpG dinucleotides has been implicated in the regulation of gene expression in vertebrates and, in some cases, has been shown to be causative in repression of **transcription**. We have examined whether methylation of CpG dinucleotides located within the **binding site** for a specific **transcription factor**, MLTF or USF, affects its **binding** to DNA. This HeLa cell **factor binds** to the adenovirus major late promoter (AdMLP), as well as endogenous cellular genes, and stimulates **transcription** in an in vitro assay. Synthetic **oligonucleotides** in which 5-methylcytosine replaces cytosine at specific sites were used to generate duplex DNAs, and the formation of complexes of these oligomers with MLTF was studied using a gel retardation assay. Methylation at a CpG site centrally located within the **binding site** strongly inhibited complex formation, whereas methylation at a site 6 bases away had no demonstrable effect. Methylation at the central site was also shown to inhibit specific **transcription** in vitro from the AdMLP. Methylation at the central site on only one strand caused a partial inhibition of **binding**, the effect being greater when the noncoding strand was methylated. The results indicate that in some cases, site-specific methylation may inhibit gene expression directly by blocking **binding** to DNA of **factors** required for **optimal transcription**. Along with other recent findings, they suggest an interplay between DNA methylation and **transcription factors** in the regulation of gene expression.

6/3,AB/35 (Item 1 from file: 5)

DIALOG(R)File 5:Biosis Previews(R)

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09286665 BIOSIS NO.: 199497295035

Random **oligonucleotide binding site optimization**

and non-nucleosomal target isolation for the **transcription factor** GATA-1.

AUTHOR: Gray Todd A(a); Collins Francis S

AUTHOR ADDRESS: (a)Prog. Cellular Molecular Biol., Univ. Michigan Sch. Med., Ann Arbor, MI 48109-0650**USA

JOURNAL: Journal of Cellular Biochemistry Supplement 0 (18C):p177
1994

CONFERENCE/MEETING: Keystone Symposium on Tumor Suppressor Genes Taos, New Mexico, USA February 13-20, 1994

ISSN: 0733-1959

RECORD TYPE: Citation

LANGUAGE: English

1994

6/3,AB/36 (Item 2 from file: 5)

DIALOG(R)File 5:Biosis Previews(R)

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07476434 BIOSIS NO.: 000091061153

DNA **BINDING** OF PURIFIED **TRANSCRIPTION FACTOR NF-KAPPAB**

AFFINITY SPECIFICITY ZINC DEPENDENCE AND DIFFERENTIAL HALF-SITE
RECOGNITION

AUTHOR: ZABEL U; SCHRECK R; BAEUERLE P A

AUTHOR ADDRESS: LABORATORY MOLECULAR BIOLOGY, GENE CENTER,
LUDWIG-MAXIMILIANS-UNIVERSITY MUNICH, AM KLOPFERSPITZ, D-8033
MARTINSRIED, W. GER.

JOURNAL: J BIOL CHEM 266 (1). 1991. 252-260. 1991

FULL JOURNAL NAME: Journal of Biological Chemistry

CODEN: JBCHA

RECORD TYPE: Abstract

LANGUAGE: ENGLISH

ABSTRACT: A rapid purification procedure for the NF-.kappa.B **transcription factor** from the cytosol of human placenta is demonstrated which exploits the insensitivity of the NF-.kappa.B .cntdot. DNA complex toward the intercalating agent chloroquine. Purified NF-.kappa.B required 100 mM KCl or NaCl and a pH of 7.5 to **optimally bind** to DNA. Equilibrium of **binding** was reached within less than 5 min in the absence of competitor DNA and after 1 h in the presence of 0.1 mg/ml poly(dI-dC). DNA **binding** of NF-.kappa.B was specifically blocked by the chelating agent 1,10-orthophenantroline and could only be reconstituted by addition of Zn²⁺. Under **optimal binding** conditions, the dissociation constant for the complex of the purified NF-.kappa.B with its most frequent cognate DNA motif 5'-GGGACTTCC-3' was in the range of 10⁻¹² to 10⁻¹³ M. Various other cis-acting .kappa.B motifs were recognized by NF-.kappa.B with lower affinities. A comparative analysis of known NF-.kappa.B-**binding sites** and competition experiments with synthetic polynucleotides and **oligonucleotides** encompassing only one half-site or single-stranded .kappa.B motifs suggested that the two DNA-**binding** monomers in the NF-.kappa.B protein complex can interact differentially with the half-sites of the decameric cognate motif.

1991

in low levels in unstimulated spleen cells and is increased by LPS treatment. This protein binds to two sites in a regulatory region of the Ia A alpha k gene, one of which contains the NF-kappa B-like binding site. DNA fragments containing these sites cross-compete for protein binding. Analysis by DNase I footprinting identified a target binding sequence, named the LPS-responsive element. Although this target sequence contains an NF-kappa B-like binding site, competition with a mutant oligonucleotide demonstrated that bases critical for NF-kappa B binding are not required for binding of the LPS-inducible protein. (ABSTRACT TRUNCATED AT 250 WORDS)

11/3,AB/34 (Item 34 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

06221542 89305516 PMID: 2787245

The human haptoglobin gene promoter: interleukin-6-responsive elements interact with a DNA-binding protein induced by interleukin-6.

Oliviero S; Cortese R

European Molecular Biology Laboratory, Heidelberg, FRG.

EMBO journal (ENGLAND) Apr 1989, 8 (4) p1145-51, ISSN

0261-4189 Journal Code: 8208664

Erratum in EMBO J 1989 Jul;8(7) 2121

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Transcription of the human haptoglobin (Hp) gene is induced by interleukin-6 (IL-6) in the human hepatoma cell line Hep3B. Cis-acting elements responsible for this response are localized within the first 186 bp of the 5'-flanking region. Site-specific mutants of the Hp promoter fused to the chloramphenicol acetyl transferase (CAT) gene were analysed by transient transfection into uninduced and IL-6-treated Hep3B cells. We identified three regions, A, B and C, defined by mutation, which are important for the IL-6 response. Band shift experiments using nuclear extracts from untreated or IL-6-treated cells revealed the presence of IL-6-inducible DNA binding activities when DNA fragments containing the A or the C sequences were used. **Competition** experiments showed that both sequences bind to the same nuclear factors. Polymers of oligonucleotides containing either the A or the C regions confer IL-6 responsiveness to a truncated SV40 promoter. The B region forms several complexes with specific DNA-binding proteins different from those which bind to the A and C region. The B region complexes are identical in nuclear extracts from IL-6-treated and untreated cells. While important for IL-6 induction in the context of the haptoglobin promoter, the B site does not confer IL-6 inducibility to the SV40 promoter. Our results indicate that the IL-6 response of the haptoglobin promoter is dependent on the presence of multiple, partly redundant, cis-acting elements.

11/3,AB/35 (Item 35 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

06167898 89251615 PMID: 2721502

Overlapping sites for constitutive and induced DNA binding factors involved in interferon-stimulated transcription.

Dale T C; Rosen J M; Guille M J; Lewin A R; Porter A G; Kerr I M; Stark G R

Imperial Cancer Research Fund Laboratories, London, UK.

EMBO journal (ENGLAND) Mar 1989, 8 (3) p831-9, ISSN 0261-4189
Journal Code: 8208664

Document type: Journal Article

Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

A 14 bp interferon (IFN)-stimulated response element (ISRE) from 6-16, a human gene regulated by alpha-IFN, confers IFN inducibility on a heterologous thymidine kinase promoter. A 39 bp double-stranded oligonucleotide corresponding to a 5' region of 6-16 which includes the ISRE competes for factors required for gene expression by alpha-IFN in transfected cells and a single base change (A-11 to C) within the ISRE (GGGAAAATGAACT) abolishes this competition. Band-shift assays performed with whole-cell extracts and the 39 bp oligonucleotide reveal specific complexes formed by rapidly induced and constitutive factors, both of which fail to bind to the A-11 to C oligonucleotide. A detailed footprinting analysis reveals that these two types of factors bind to overlapping sites within the ISRE, but in very different ways. These data were used to design oligonucleotides which decreased the formation of the inducible complex without affecting the constitutive one. Changes at the 5' margin of the ISRE and upstream of it markedly decrease formation of the induced but not the constitutive complex and also abolish the ability of the 39 bp sequence to function as an inducible enhancer with the thymidine kinase promoter. Thus, induction of 6-16 transcription in IFN-treated cells is likely to be stimulated by binding of the induced factor to the ISRE and upstream sequences, while the subsequent suppression of transcription may involve competition for the ISRE by the other class of factors.

11/3,AB/36 (Item 36 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

06137801 89219068 PMID: 2540425

Negative control region at the 5' end of murine leukemia virus long terminal repeats.

Flanagan J R; Krieg A M; Max E E; Khan A S
Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892.

Molecular and cellular biology (UNITED STATES) Feb 1989, 9 (2)
p739-46, ISSN 0270-7306 Journal Code: 8109087

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Using in vitro protein binding and in vivo functional studies, we have identified novel regulatory sequences near the 5' end of murine leukemia virus (MuLV) long terminal repeats (LTRs). These sequences are highly conserved in all MuLV LTRs as well as in feline leukemia virus and gibbon ape leukemia virus LTRs. In this upstream conserved region (UCR), gel retardation assays detected two overlapping but distinct binding sites (UCR-U and UCR-L) for nuclear proteins (UCRF-U and UCRF-L). Three lines of evidence suggest a negative regulatory role for the UCR in viral transcription: (i) an inverse correlation was found between MuLV transcripts and nuclear proteins binding the UCR in the spleens of five different mouse strains; (ii) in vivo treatment of NFS mice with lipopolysaccharide resulted in the induction of splenic viral transcripts and the concomitant disappearance of UCR-binding proteins; and (iii) in mouse L cells transfected with an MuLV LTR linked to the chloramphenicol acetyltransferase (CAT) gene, cotransfected UCR oligonucleotides increased CAT expression, presumably by competing for inhibitory trans-acting factors.

11/3,AB/37 (Item 37 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

06115362 89199737 PMID: 2539489

Transcriptionally active immediate-early protein of pseudorabies virus binds to specific sites on class II gene promoters.

Cromlish W A; Abmayr S M; Workman J L; Horikoshi M; Roeder R G
Laboratory of Biochemistry and Molecular Biology, Rockefeller University,
New York, New York 10021-6399.

Journal of virology (UNITED STATES) May 1989, 63 (5) p1869-76,
ISSN 0022-538X Journal Code: 0113724

Contract/Grant No.: CA-42567; CA; NCI; GM11077; GM; NIGMS; T32 A107233;
PHS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

In the presence of partially purified pseudorabies virus immediate-early protein, multiple sites of DNase I protection were observed on the adenovirus major late and human hsp 70 promoters. Southwestern (DNA-protein blot) analysis demonstrated that the immediate-early protein bound directly to the sequences contained in these sites. These sequences share only limited homology, differ in their affinities for the immediate-early protein, and are located at different positions on these two promoters. In addition, the site-specific binding of a temperature-sensitive immediate-early protein was eliminated by the same heat treatment which eliminates its transcriptional activating function, whereas the binding of the wild-type protein was unaffected by heat treatment. Thus, site-specific binding requires a functionally active immediate-early protein. Furthermore, immediate-early-protein-dependent in vitro transcription from the major late promoter was preferentially inhibited by oligonucleotides which are homologous to the high-affinity binding sites on the major late or hsp 70 promoters. These observations suggest that transcriptional stimulation by the immediate-early protein involves binding to cis-acting elements.

11/3,AB/38 (Item 38 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

05833531 88257120 PMID: 2454922

The gonadotropin alpha-gene contains multiple protein binding domains that interact to modulate basal and cAMP-responsive transcription.

Jameson J L; Jaffe R C; Deutsch P J; Albanese C; Habener J F

Thyroid Unit, Massachusetts General Hospital, Boston 02114.

Journal of biological chemistry (UNITED STATES) Jul 15 1988, 263

(20) p9879-86, ISSN 0021-9258 Journal Code: 2985121R

Contract/Grant No.: AM25532; AM; NIADDK; HD23262; HD; NICHD

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

DNA sequences that modulate basal and cAMP-stimulated transcription are located within the initial 169 base pairs of the alpha-gene 5'-flanking sequence. Using DNase I protection analyses and gel-mobility shift assays, we examined in vitro the domains in the human alpha-gene 5'-flanking sequence that bind nuclear proteins extracted from JEG-3 choriocarcinoma cells. DNase I protection studies of the sequences between -236 and -100 demonstrate two major protected regions: -178 to -156 corresponding to an upstream regulatory element (URE) and -146 to -112 corresponding to 18-base pair repeated sequences that contain cAMP-responsive enhancers (CREs). Nuclear proteins extracted from JEG-3 choriocarcinoma cells bind specifically to oligonucleotides corresponding to both the URE and CRE domains as well as to a downstream domain (-99 to -72) that contains consensus CCAAT motifs on both the sense

and antisense strands. **Binding** to a DNA fragment (-236 to -100) that contains both the URE and CRE domains was 10-fold more effective than that using either fragment alone. **Binding** to this multisite DNA fragment is readily disrupted using the URE sequence, but not the CRE sequence as a **competitor**, suggesting that the URE **binding factor** may stabilize DNA-protein interactions in these adjacent complexes. The amount of protein **binding** to each of the alpha-gene 5'-flanking domains was unaffected by **treatment** with 8-bromo-cAMP. These studies indicate that there are multiple adjacent protein **binding** domains in human alpha-gene 5'-flanking sequence that correspond to cis-acting regulatory elements including an upstream element that activates basal expression, repeated cAMP-response elements, and a downstream sequence containing consensus CCAAT box elements. Interactions between regulatory domains facilitate protein **binding** and synergistically stimulate alpha-gene **transcription**.

11/3,AB/39 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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10003179 BIOSIS NO.: 199598458097
Biological Activity in the Repopulating Rat Spermatocyte After the
Withdrawal of Gossypol **Treatment**. VI. Alteration in Nuclear
Factors for Interaction With Histone Gene Promoter.
AUTHOR: Tseng C S(a); Yang N Y; Chen Y
AUTHOR ADDRESS: (a)Coll. Vet. Med., North Carolina State Univ., Dep. Anat.,
Physiol. Sci. Radiol., 4700 Hillsboroug**USA
JOURNAL: Contraception 52 (2):p129-135 1995
ISSN: 0010-7824
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: This article reports the effects of gossypol at the genomic level in rat spermatogenic cells. After gossypol **treatment** for various times (8, 12, and 19 weeks), the spermatogonial cells were allowed to rest for 2 to 4 weeks. The function of histone H-4 gene promoter (H-4GP) in the repopulating pachytene spermatocytes (RPS) was investigated. The sequences of the **oligonucleotides** for the H-4GP **binding sites** 1 and 2 were synthesized by an ABI-392 DNA synthesizer. RPS and the control pachytene spermatocytes (CPS) were obtained by centrifugal elutriation and subsequently they were used for the preparation of nuclear protein extracts (NPE). The NPE interaction with the DNA fragment of site 1 or 2 was studied by an electrophoresis mobility shift assay (EMSA). EMSA with NPE-CPS revealed ten major gel shift bands for site 1 and 2. The presence of extra unlabeled DNA fragments **competed** with 6 of the bands. After 2 to 4 weeks recovery from 8, 12, and 19 weeks of gossypol **treatment**, NPE-RPS failed to shift four bands (b through e) in site 1. These results suggested that gossypol **treatment** affected the **transcription factors** for interaction with site 1. On the contrary, no effect was demonstrated in NPE that interacted with site 2. Furthermore, gossypol **treatment** did not change the nucleotide sequence in the H-4GP site 1 and 2.

1995

11/3,AB/40 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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07432489 BIOSIS NO.: 000091038478
INTERLEUKIN-1-MEDIATED ENHANCEMENT OF MOUSE **FACTOR B** GENE EXPRESSION

VIA NF-K-B-LIKE HEPATOMA NUCLEAR **FACTOR**

AUTHOR: NONAKA M; HUANG Z-M

AUTHOR ADDRESS: DEP. IMMUNOL., CANCER RES. INST., KANAZAWA UNIV., KANAZAWA, JAPAN 920.

JOURNAL: MOL CELL BIOL 10 (12). 1990. 6283-6289. 1990

FULL JOURNAL NAME: Molecular and Cellular Biology

CODEN: MCEBD

RECORD TYPE: Abstract

LANGUAGE: ENGLISH

ABSTRACT: Complement **factor B**, a serine protease playing a pivotal role in alternative pathway activation, is an acute-phase plasma protein. Previous studies have revealed that interleukin-1 (IL-1) mediates, at least in part, the acute-phase induction of **factor B** expression and that the IL-1-responsive element resides in the region between -553 and -478 relative to the **transcription** initiation site of the mouse **factor B** gene. In this paper, we demonstrate a specific **binding site** for a nuclear **factor** of human hepatoma HepG2 cells in this region of the **factor B** gene, using gel shift and methylation interference analysis. The nucleotide sequence of the **binding site** is closely similar to the NF.kappa.B or H2TF1 **binding** motif. The **binding** activity of HepG2 showed very similar specificity to that of NF.kappa.B or H2TF1, as shown by a **competition binding** assay, and was induced by IL-1.alpha. **treatment**. A synthetic **oligonucleotide** corresponding to this **binding site**, as well as a similar sequence found in another class III complement C4 gene, conferred IL-1 responsiveness on the minimal **factor B** promoter. In contrast, a mutated **oligonucleotide** that could not **bind** to the HepG2 nuclear **factor** did not confer IL-1 responsiveness. These results suggest that IL-1 induces **factor B** expression via NF.kappa.B or a closely related **factor** in hepatocyte nuclei.

1990

? ds

Set	Items	Description
S1	84600	PY<1998 AND TRANSCRIPTION AND FACTOR?
S2	4529	S1 AND OLIGONUCLEOTID?
S3	3397	S2 AND BIND?
S4	1566	S3 AND (BINDING (W) SITE?)
S5	47	S4 AND OPTIM?
S6	36	RD (unique items)
S7	491	S4 AND COMPET?
S8	32	S7 AND VECTOR?
S9	20	RD (unique items)
S10	54	S7 AND (ADMINISTER? OR TREAT?)
S11	40	RD (unique items)

? s s1 and malignant?

	84600	S1
	278128	MALIGNANT?
S12	1016	S1 AND MALIGNANT?

? s s12 and treat?

	1016	S12
	3346815	TREAT?
S13	189	S12 AND TREAT?

? s s13 not s11

	189	S13
	40	S11
S14	189	S13 NOT S11

? s s14 not s10

	189	S14
	54	S10
S15	189	S14 NOT S10

? s s15 not s8

	189	S15
	32	S8
S16	189	S15 NOT S8

? s s16 not s5

	189	S16
	47	S5
S17	189	S16 NOT S5

? rd

...examined 50 records (50)
...examined 50 records (100)
...examined 50 records (150)
...completed examining records
S18 134 RD (unique items)

? t s18/3,ab/all

18/3,AB/1 (Item 1 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

10687162 20234323 PMID: 10771828

The causes of cancer: implications for prevention and **treatment**.

Madhukar B V; Trosko J E

Department of Pediatrics and Human Development, College of Human
Medicine, Michigan State University, East Lansing, USA.

Indian journal of pediatrics (INDIA) Mar-Apr 1997, 64 (2)

p131-41, ISSN 0019-5456 Journal Code: 0417442

Contract/Grant No.: CA-21104; CA; NCI; ES-04911; ES; NIEHS

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The final clinical manifestation of cancer is a result of complex series
of changes in a single cell. This review summarizes some of the new
concepts and hypotheses that explain the evolution of cancers. The emphasis

is on cancer as a disease of the stem cells within a tissue that undergo initiation as a result of mutational insult to one or more genes that are critical for cell growth. During the second stage (promotion stage) the initiated cells acquire proliferative capacity due to epigenetic changes, i.e., altered expression of genes whose products play a central role in signal transduction. This requires continued exposure to agents and events causing such changes. This stage is, therefore, reversible and the various components of this stage are central targets for the development of mechanism based anti-cancer drugs. During the stage of progression, the neoplastic lesions acquire additional genetic alterations and become clinically manifestable **malignant** neoplasms. At the biochemical and molecular level, neoplastic transformation involves aberrations in the expression and regulation of oncogenes, tumor suppression genes, **transcription factors** and components of the cell signal transduction cascades. The understanding of the various cellular biochemical and molecular events that metamorphose a normal cell into a cancer cell is central to the development of rational new drugs that are targeted against the various components. Such drugs in combination with the conventional chemotherapeutic agents that are currently used, provide a more effective control of cancer without the risk of toxic side effects.

18/3,AB/2 (Item 2 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

10129715 99111016 PMID: 9815767

Interferon induces thymidine phosphorylase/platelet-derived endothelial cell growth **factor** expression in vivo.

Makower D; Wadler S; Haynes H; Schwartz E L

Department of Oncology, Albert Einstein Cancer Center, Albert Einstein College of Medicine, Bronx, New York 10467, USA.

Clinical cancer research : an official journal of the American Association for Cancer Research (UNITED STATES) Jun 1997, 3 (6)

p923-9, ISSN 1078-0432 Journal Code: 9502500

Contract/Grant No.: CA13330; CA; NCI; CA55442; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The enzyme/cytokine thymidine phosphorylase/platelet-derived endothelial cell growth **factor** (TP/PD-ECGF) has diverse functions within cells, including the regulation of steady-state thymidine levels, the conversion of the cancer chemotherapeutic agent 5-fluorouracil (FUra) to an active metabolite, and the mediation of angiogenesis in normal and **malignant** cells. Although the levels of TP/PD-ECGF vary substantially among different tissues and are generally found to be elevated in tumors, little is known about the control of its expression in vivo in humans. In this study, peripheral blood mononuclear cells were obtained from patients prior to and during **treatment** with IFN and FUra and analyzed for TP/PD-ECGF expression. Sixteen of 21 patients (76%) exhibited an average 3-4-fold increase of TP/PD-ECGF protein levels after **treatment** with either IFN-alpha or-beta, with the remaining patients having either a decrease (four patients) or no change (one patient) at the sampling times examined. Expression in vivo increased rapidly within 1-2 h of IFN **treatment** and remained elevated for up to 48 h after its administration. The increase in TP/PD-ECGF protein was accompanied by a concomitant increase in TP/PD-ECGF mRNA levels. TP/PD-ECGF mRNA expression in cells in vitro was induced by IFN but not by pharmacologically relevant concentrations of FUra, suggesting that the IFN was responsible for the induction seen in the patients. This study demonstrates that IFN induces TP/PD-ECGF expression in vivo by regulation of the level of mRNA expression.

18/3,AB/3 (Item 3 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

09851346 98291183 PMID: 9627713

Human melanoma genetics.

Kamb A

Myriad Genetics, Inc., Salt Lake City, UT 84108, USA.

Journal of investigative dermatology. Symposium proceedings / the Society for Investigative Dermatology, Inc. and European Society for Dermatological Research (UNITED STATES) Apr 1996, 1 (2) p177-82, ISSN

1087-0024 Journal Code: 9609059

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Melanoma is an important human cancer, the etiology of which has been the subject of much study. Recently a gene for familial melanoma, MLM, has been mapped and isolated. This gene encodes the cell-cycle regulator p16 and is mutated in a variety of sporadic human cancers in addition to melanoma. The isolation of MLM answers some questions in the area of melanoma biology, but raises others. Identification of p16 and other genes that contribute to melanoma development may be viewed as one step in the attempt to understand, diagnose, and **treat** this **malignant** disease.

18/3,AB/4 (Item 4 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

09673923 98095442 PMID: 9433644

Retinoids and chemoprevention of aerodigestive tract cancers.

Lotan R

Department of Tumor Biology, University of Texas M.D. Anderson Cancer Center, Houston, USA.

Cancer and metastasis reviews (UNITED STATES) Sep-Dec 1997, 16

(3-4) p349-56, ISSN 0891-9992 Journal Code: 8605731

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Natural and synthetic vitamin A metabolites and analogs (retinoids) were found to suppress head and neck and lung carcinogenesis in animal models and inhibit carcinogenesis in individuals with premalignant lesions and a high risk to develop cancer of the aerodigestive tract. Likewise, retinoids prevent the development of second primary cancers in head and neck and lung cancer patients who had been **treated** for the first primary. These effects are thought to result from changes in the expression of genes that regulate cell growth and differentiation. Most of the effects of retinoids on gene expression are mediated by nuclear retinoic acid receptors RARs (alpha, beta, and gamma) and retinoid X receptors (RXR alpha, beta, and gamma), which function as retinoid-activated **transcription factors**. Like vitamin A deficiency, alterations in receptor expression or function could interfere with the retinoid signaling pathway and thereby enhance cancer development even in vitamin A sufficient individuals. We found that the expression of RAR beta was suppressed in more than 50% of oral and lung premalignant lesions in individuals without cancer (e.g., oral leukoplakia and squamous metaplasia), in dysplastic lesions adjacent to cancer, and in **malignant** oral and lung carcinomas. The expression of the other receptors was not different among normal, dysplastic, and **malignant** oral tissues. However, the expression of RAR gamma and RXR beta was somewhat decreased in lung cancers. These results show that RAR beta expression is lost at early stages of carcinogenesis in the aerodigestive tract and support the hypothesis that the loss of RAR beta expression may facilitate the development of some of these cancers.

18/3,AB/5 (Item 5 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09657427 98095062 PMID: 9433477

Differential expression of human ferritin H chain gene in immortal human breast epithelial MCF-10F cells.

Higgy N A; Salicioni A M; Russo I H; Zhang P L; Russo J
Breast Cancer Research Laboratory, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111, USA.

Molecular carcinogenesis (UNITED STATES) Dec 1997, 20 (4)
p332-9, ISSN 0899-1987 Journal Code: 8811105

Contract/Grant No.: R01-CA67238; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subtractive hybridization was used to isolate genes expressed uniquely in the immortalized human breast epithelial cell (HBEC) line MCF-10F and not in the mortal HBEC line S-130, from which MCF-10F cells were derived. We identified a 233-bp cDNA that was expressed in MCF-10F cells and not in their mortal counterpart S-130 cells. Sequence comparison with the GenBank database revealed that the cDNA was identical to the gene encoding human ferritin heavy H chain. Northern blot analysis using the isolated cDNA as a probe showed a differentially expressed 1.1-kb transcript of ferritin H in total RNA from the immortal MCF-10F cells, MCF-10F cells treated with the chemical carcinogens 7,12-dimethylbenz[a]anthracene and benzo[a]pyrene, and the breast cancer cell lines MCF-7, HBL-100, T-47D, and BT-20. No ferritin H transcript was detected in the mortal line S-130 or in other primary HBEC cultures. Increased levels of mRNA transcript signals were also detected in total RNA from breast cancer tissue samples. Tissue with ductal hyperplasia had higher expression levels than normal adjacent mammary tissue. In situ hybridization showed high levels of ferritin H transcript in mammary tissue areas with ductal hyperplasia, carcinoma in situ, and infiltrating ductal carcinoma. This is the first report of the differential expression and upregulation of human ferritin H chain gene in immortal HBECs. It may be an important factor in the process of immortalization, possibly an early stage of malignant transformation of HBECs, providing cells with iron necessary for growth and clonal expansion. Also, ferritin iron, once released, may increase the level of reactive iron, leading to an increase in oxygen free-radical generation, oxidative DNA damage, and mutation.

18/3,AB/6 (Item 6 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09655002 98087166 PMID: 9427294

Lactoferrin expression in mammary epithelial cells is mediated by changes in cell shape and actin cytoskeleton.

Close M J; Howlett A R; Roskelley C D; Desprez P Y; Bailey N; Rowning B; Teng C T; Stampfer M R; Yaswen P

Division of Life Sciences, University of California, Berkeley 94720, USA.

Journal of cell science (ENGLAND) Nov 1997, 110 (Pt 22)
p2861-71, ISSN 0021-9533 Journal Code: 0052457

Contract/Grant No.: CA-24844; CA; NCI; CA-57621; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Lactoferrin is a secreted iron binding protein which is expressed during normal functional development of mammary epithelium. Murine mammary epithelial cell lines competent for milk protein expression were used to identify microenvironmental factors that regulate lactoferrin

expression. While lactoferrin was not expressed in adherent monolayer cultures under standard subconfluent conditions on plastic, lactoferrin mRNA and protein steadily accumulated when the cells aggregated to form spheroids on a reconstituted basement membrane gel. However, unlike other milk proteins such as beta-casein, lactoferrin expression was also induced at high cell density in the absence of exogenously added basement membrane or prolactin. These results led us to examine whether changes in cell growth, cell-cell interactions and/or cell shape were responsible for regulation of lactoferrin gene expression. Rounded, non-proliferating cells in suspension in serum-free medium expressed lactoferrin even as single cells. Conversely, lactoferrin expression could be inhibited in non-proliferative cells in serum-free medium by maintaining them in contact with an air-dried extracellular matrix which caused the cells to retain flat, spread morphologies. These findings indicated that cessation of cell growth was not sufficient, that cell-cell interactions were not required, and that cell culture conditions which minimize cell spreading may be important in maintaining lactoferrin expression. Additional data supporting this latter concept were generated by **treating** spread cells with cytochalasin D. The resulting disruption of microfilament assembly induced both cell rounding and lactoferrin expression. Shape-dependent regulation of lactoferrin mRNA was both transcriptional and post-transcriptional. Surprisingly, **treatment** of rounded cells with a **transcription** inhibitor, actinomycin D, produced a stabilization of lactoferrin mRNA, suggesting that **transcription** of an unstable **factor** is required for degradation of lactoferrin mRNA. Importantly, lactoferrin mRNA expression was regulated similarly in early passage normal human mammary epithelial cells. In vivo, the changing extracellular matrix components of the mammary gland during different stages of normal and abnormal growth and differentiation may provide different physical constraints on the configurations of cell surface molecules. These physical constraints may be communicated to the cell interior through mechanical changes in the cytoskeleton. Unlike beta-casein whose expression is upregulated by specific integrin-mediated signals, lactoferrin may be representative of a class of proteins synthesized in the mammary gland using basal transcriptional and translational machinery. The suppression of lactoferrin expression that is observed in monolayer culture and in **malignant** tissues may reflect inappropriate cell shapes and cytoskeletal structures that are manifested under these conditions.

18/3,AB/7 (Item 7 from file: 155)
 DIALOG(R) File 155:MEDLINE(R)

09613926 98040269 PMID: 9374113

Identification of early growth response gene-1 (Egr-1) as a phorbol myristate acetate-induced gene in lung cancer cells by differential mRNA display.

You L; Jakowlew S B
 National Cancer Institute, Medicine Branch, Rockville, Maryland 20850, USA.

American journal of respiratory cell and molecular biology (UNITED STATES)
) Nov 1997, 17 (5) p617-24, ISSN 1044-1549 Journal Code:
 8917225

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Cellular regulatory genes including **transcription factors** may play an important role in the induction, maintenance, and progression of lung cancer. These regulatory genes are inducible by various mitogenic stimuli including phorbol myristate acetate (PMA). The differential mRNA display method was used to identify potential early response genes regulated by PMA in non-small cell lung cancer (NSCLC) cell lines. Using this technique, several cDNA fragments were found to be potentially

differentially regulated by PMA in the squamous NSCLC cell line NCI-H157. One of these cDNA fragments of approximately 100 bp was determined to be differentially induced by at least 30-fold by PMA by northern blot analysis and to hybridize to a single 3.4 kb mRNA species. This cDNA fragment was cloned, sequenced, and identified to be identical to a portion of the 3'-untranslated region of the human early growth response gene-1 (Egr-1). Using Egr-1 cDNA as a probe, it was demonstrated that PMA induces Egr-1 mRNA expression in at least three other NSCLC cells as well. In addition, PMA caused a transient increase in expression of the Egr-1 transcript reaching a maximum level by 1 h before decreasing in NCI-H157 and three other types of NSCLC cells. **Treatment** of these NSCLC cells with TGF-beta1 showed a transient increase in Egr-1 mRNA similar to PMA which also reached a maximum level after 1 h. Normal human bronchial epithelial (NHBE) cells also showed a rapid, transient increase in expression of Egr-1 mRNA after **treatment** with PMA. In contrast, **treatment** of NHBE cells with TGF-beta1 showed that expression of Egr-1 mRNA increased by 1 h but reached a maximum level only after 6 h. These results indicate that both PMA and TGF-beta1 can induce Egr-1 mRNA expression in NSCLC cells and NHBE cells; however, while PMA induces Egr-1 mRNA similarly in both cell types, TGF-beta1 induces Egr-1 mRNA expression more rapidly and more transiently in NSCLC cells than in NHBE cells. Our results suggest that Egr-1 may play different roles in response to mitogens in normal and **malignant** lung cells.

18/3,AB/8 (Item 8 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09592958 98030788 PMID: 9364213

bcl-2 enhancement of **malignant** transformation in mouse epidermal JB6 cells.

Amstad P A; Liu H; Ichimiya M; Chang S; Berezesky I K; Trump B F
Department of Pathology, University of Maryland, Baltimore 21201, USA.

Molecular carcinogenesis (UNITED STATES) Oct 1997, 20 (2)
p231-9, ISSN 0899-1987 Journal Code: 8811105

Contract/Grant No.: DK15440; DK; NIDDK

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Increased bcl-2 expression is a common feature of many types of human malignancies, which implies that bcl-2 plays an important role in tumorigenesis. To better understand the molecular mechanisms of bcl-2-induced oncogenesis, we examined the effects of bcl-2 expression on transformation of mouse epidermal JB6 cells induced by the tumor promoter 12-O-tetradecanoylphorbol-13 acetate (TPA). Promotion-sensitive JB6 clone41 cells were transfected with the bcl-2-containing expression vector pD5-neo/bcl-2, and the soft agar growth of bcl-2-transfected cells and control cells were compared. bcl-2 overexpression in JB6 clone41 cells caused a TPA-induced soft-agar growth fivefold greater than the growth of nontransfected or vector-transfected (neo control) cells. bcl-2 expression in the absence of TPA did not lead to colony formation in soft agar. Because the level of the **transcription factor** activator protein 1 (AP-1) has been shown to be critical for the responsiveness of JB6 cells to TPA-induced transformation, we compared c-jun and c-fos expression as well as the AP-1-binding activity and the AP-1-mediated transactivation of the reporter construct TRE-CAT between bcl-2-expressing cells and control cells. When compared with control cells, bcl-2-transfected cells expressed significantly more c-fos but not c-jun after TPA **treatment**. Furthermore, the levels of AP-1 and AP-1-induced transactivation of TRE-CAT were greater in bcl-2-transfected cells than in control cells after TPA **treatment**. These results showed that bcl-2 cooperates with a tumor promoter such as TPA in the induction of **malignant** transformation in mouse epidermal cells and that bcl-2 enhances soft-agar growth by

stimulating signaling pathways that led to increased AP-1 expression.

18/3,AB/9 (Item 9 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09542069 97452190 PMID: 9306961

Expression of human intestinal trefoil **factor** in **malignant** cells and its regulation by oestrogen in breast cancer cells.

May F E; Westley B R

Department of Pathology, Royal Victoria Infirmary, Newcastle upon Tyne, U.K.

Journal of pathology (ENGLAND) Aug 1997, 182 (4) p404-13,
ISSN 0022-3417 Journal Code: 0204634

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Human intestinal trefoil **factor** (hITF) is a small cysteine-rich protein expressed in the gastrointestinal (GI) tract. Its sequence is related to that of other trefoil peptides including the pNR-2/ps2 protein, which is regulated by oestrogen in breast cancer. This study was designed to investigate whether hITF is expressed in human carcinoma cells. cDNA was obtained by reverse **transcription**-polymerase chain reaction (RT-PCR) of gastric mucosal RNA and sequenced, establishing that this mRNA is expressed in the stomach. Expression of hITF was detected in a proportion of cell lines derived from malignancies of the GI tract, in hepatocellular carcinoma cells, and at highest levels in a small cell lung carcinoma cell line. Amongst breast cancer cell lines, it was expressed in all the oestrogen-responsive but in none of the oestrogen-nonresponsive breast cancer cell lines. The possibility that hITF expression in breast cells is controlled by oestradiol was then tested. Oestradiol **treatment** increased hITF expression between three- and ten-fold in the oestrogen-responsive breast cancer cell lines, demonstrating that, like pNR-2/ps2, hITF is regulated by oestrogen in breast cancer cells. Tamoxifen inhibited the induction of hITF expression by oestradiol but tamoxifen alone was a partial oestrogen agonist for hITF expression. These results show that hITF is expressed, sometimes ectopically, in several human malignancies, which suggests that trefoil peptides may have a more general role in tumourigenesis than hitherto appreciated. That the expression of hITF is regulated by oestrogen in breast cancer cells suggests that hITF expression may provide a novel marker for oestrogen responsiveness in breast cancer.

18/3,AB/10 (Item 10 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09540331 97436288 PMID: 9290955

Novel MLL-CBP fusion transcript in therapy-related chronic myelomonocytic leukemia with a t(11;16)(q23;p13) chromosome translocation.

Satake N; Ishida Y; Otoh Y; Hinohara S; Kobayashi H; Sakashita A; Maseki N; Kaneko Y

Third Clinical Department, Saitama Cancer Center Hospital, Japan.

Genes, chromosomes & cancer (UNITED STATES) Sep 1997, 20 (1)
p60-3, ISSN 1045-2257 Journal Code: 9007329

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

CBP, which is located on 16p13 and encodes a transcriptional adaptor/coactivator protein, has been shown to fuse by the t(8;16)(p11;p13) translocation to MOZ on 8p11 in acute myeloid leukemia. We found a t(11;16)(q23;p13) in a child with therapy-related chronic myelomonocytic

leukemia. Subsequent reverse transcriptase-polymerase chain reaction and direct sequencing analyses revealed the MLL-CBP fusion transcript in CMML cells. Because 11q23 translocations involving MLL and t(8;16) involving MOZ and CBP have been reported in therapy-related leukemias, both the MLL and CBP genes may be targets for topoisomerase II inhibitors. Accordingly, we believe that most t(11;16)-associated leukemias may develop in patients who have been **treated** with cytotoxic chemotherapy for primary **malignant** diseases.

18/3,AB/11 (Item 11 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09499910 97413638 PMID: 9270024

Expression of neurogenic basic helix-loop-helix genes in primitive neuroectodermal tumors.

Rostomily R C; Bermingham-McDonogh O; Berger M S; Tapscott S J; Reh T A; Olson J M

Department of Neurological Surgery, The University of Washington School of Medicine, Seattle 98195, USA.

Cancer research (UNITED STATES) Aug 15 1997, 57 (16) p3526-31, ISSN 0008-5472 Journal Code: 2984705R

Contract/Grant No.: NS 30304; NS; NINDS; RO1 NS 28308; NS; NINDS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The basic helix-loop-helix (bHLH) class of **transcription factors** plays a pivotal role in tissue-specific determination and differentiation. Moreover, dysregulated expression or loss of function of these **factors** contributes to leukemogenesis and solid tumor development. Neurogenesis is regulated by genes of the NEUROD/atonal and ACHAETE SCUTE families. We analyzed expression of human NEUROD1, NEUROD2, NEUROD3, and ACHAETE SCUTE 1 (HASH1) in cerebellar and cerebral primitive neuroectodermal tumors (PNETs), gliomas, and cell lines derived from a variety of neuroectodermal tumors by Northern analysis and in situ hybridization. NEUROD1 was expressed in each of the 12 medulloblastoma specimens, whereas NEUROD2 and NEUROD3/neurogenin were expressed in partly overlapping subsets of medulloblastomas. All of the tumors that presented with distant metastases expressed NEUROD3. The only other NEUROD3-positive tumor progressed early in **treatment**. Human ACHAETE SCUTE homologue (HASH1) was not expressed in medulloblastomas (infratentorial PNETs) but was expressed in three of five supratentorial PNETs. Neuroectodermal tumor cell lines derived from other sites (e.g., neuroblastoma and retinoblastoma) expressed NeuroD and ACHAETE SCUTE family members. No NEUROD message was detected in glial tumors or cell lines. Neurogenic bHLH **transcription factor** expression patterns suggest that specific family members may contribute to or reflect biological differences that arise during **malignant** transformation.

18/3,AB/12 (Item 12 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09499003 97396501 PMID: 9252692

MDA468 growth inhibition by EGF is associated with the induction of the cyclin-dependent kinase inhibitor p21WAF1.

Xie W; Su K; Wang D; Paterson A J; Kudlow J E

Department of Cell Biology and Medicine, University of Alabama at Birmingham 35294, USA.

Anticancer research (GREECE) Jul-Aug 1997, 17 (4A) p2627-33, ISSN 0250-7005 Journal Code: 8102988

Contract/Grant No.: DK48882; DK; NIDDK

Document type: Journal Article

Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Epidermal growth factor (EGF) usually stimulates the proliferation of a variety of normal and **malignant** cells. In contrast, MDA468, a human breast cancer cell line with a very high number of EGF receptors, is growth inhibited in response to concentrations of EGF that stimulate most other cells. The purpose of this study was to elucidate the cellular mechanisms involved in EGF-induced growth inhibition. EGF **treatment** stimulated the sustained expression of the cyclin-dependent kinase (CDK) inhibitor p21WAF1. The p21WAF1 induction in EGF-**treated** MDA468 cells is probably p53-independent since these cells contain no active p53. The promoter for p21WAF1 gene contains binding sites for signal transducer and activator of **transcription** (STAT) and EGF is known to activate members of this family of **transcription factors**. Using electrophoretic mobility shift assays (EMSA), we found that EGF activates STAT1 and STAT3 in the MDA468 cells. These activated STATs specifically recognized the three conserved STAT-responsive elements in the p21WAF1 gene promoter, suggesting that STATs may be responsible for the p21WAF1 induction by EGF in MDA468 cells. The sustained rise in p21WAF1 in response to EGF is proposed to be a means of growth inhibition in these cells.

18/3,AB/13 (Item 13 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09419508 97329528 PMID: 9186002

Retinoic acid induces signal transducer and activator of **transcription** (STAT) 1, STAT2, and p48 expression in myeloid leukemia cells and enhances their responsiveness to interferons.

Matikainen S; Ronni T; Lehtonen A; Sareneva T; Melen K; Nordling S; Levy D E; Julkunen I

National Public Health Institute, Helsinki, Finland.

Cell growth & differentiation : the molecular biology journal of the American Association for Cancer Research (UNITED STATES) Jun 1997,

8 (6) p687-98, ISSN 1044-9523 Journal Code: 9100024

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

IFNs are antiproliferative cytokines that have growth-inhibitory effects on various normal and **malignant** cells. Therefore, they have been used in the **treatment** of certain forms of cancer, such as chronic myelogenous leukemia and hairy cell leukemia. However, there is little evidence that IFNs would be effective in the **treatment** of acute myelogenous leukemia, and molecular mechanisms underlying IFN unresponsiveness have not been clarified. Here we have studied the activation and induction of IFN-specific **transcription factors**

signal transducer and activator of **transcription** (STAT) 1, STAT2, and p48 in all-trans-retinoic acid (ATRA)-differentiated myeloid leukemia cells using promyelocytic NB4, myeloblastic HL-60, and monoblastic U937 cells as model systems. These cells respond to ATRA by growth inhibition and differentiation. We show that in undifferentiated NB4 cells, 2',5'-oligoadenylate synthetase and MxB gene expression is not activated by IFN-alpha, possibly due to a relative lack of signaling molecules, especially p48 protein. However, during ATRA-induced differentiation, steady-state STAT1, STAT2, and especially p48 mRNA and corresponding protein levels were elevated both in NB4 and U937 cells, apparently correlating to an enhanced responsiveness of these cells to IFNs. ATRA **treatment** of NB4 cells sensitized them to IFN action as seen by increased IFN-gamma activation site DNA-binding activity or by efficient formation of IFN-alpha-specific ISGF3 complex and subsequent oligoadenylate synthetase and MxB gene expression. Lack of p48 expression could be one of the mechanisms of promyelocytic leukemia cell escape from growth-inhibitory

effects of IFN-alpha.

18/3,AB/14 (Item 14 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09417383 97309767 PMID: 9167178

Prognostic significance of nm23 protein expression in **malignant** melanoma. An immunohistochemical study.

van den Oord J J; Maes A; Stas M; Nuyts J; De Wever I; De Wolf-Peeters C
Department of Pathology II, University Hospital St Rafael, KUL, Leuven, Belgium.

Melanoma research (ENGLAND) Apr 1997, 7 (2) p121-8, ISSN 0960-8931 Journal Code: 9109623

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The NM23 genes, encoding for the red blood cell nucleoside diphosphate kinases A and B, have been found to serve as metastasis-suppressor genes in experimental animal models of tumour progression, and in some, but not all cancers in man. To investigate the role of NM23 in the progression of human **malignant** melanoma, we studied the expression and distribution of the nm23 protein with a sensitive immunohistochemical technique and a well-characterized monoclonal antibody in 41 benign pigment cell lesions and 71 uniformly **treated malignant** melanomas with a long follow up-up. In benign naevi, the junctional nests frequently expressed nm23 protein, whereas the immunoreactivity tended to decrease when the lesions matured. All **malignant** melanomas expressed nm23 protein in their vertical and/or radial growth phases, and the immunoreactivity tended to increase towards the deeper parts of the lesion. No relation was found between nm23 expression and patient outcome. In addition, nm23 was found in activated lymphoid cells, and this feature was significantly associated with a brisk lymphocytic stroma response in **malignant** melanomas. Our data are at variance with previous mRNA studies on **malignant** melanoma, and indicate that routine immunohistochemical analysis for nm23 protein on paraffin-embedded tumour tissue cannot reliably be used as a prognostic marker for patients suffering from **malignant** melanoma. In contrast, our findings suggest that the nm23 protein in pigment cell lesions is related to the proliferative or activated state of pigment cells, rather than to their metastatic potential.

18/3,AB/15 (Item 15 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09396648 97300191 PMID: 9155263

Phthalocyanine photodynamic therapy: disparate effects of pharmacologic inhibitors on cutaneous photosensitivity and on tumor regression.

Anderson C; Hrabovsky S; McKinley Y; Tubesing K; Tang H P; Dunbar R; Mukhtar H; Elmetts C A

Department of Dermatology, Case Western Reserve University, Cleveland, OH, USA.

Photochemistry and photobiology (UNITED STATES) May 1997, 65

(5) p895-901, ISSN 0031-8655 Journal Code: 0376425

Contract/Grant No.: AR39750; AR; NIAMS; CA48735; CA; NCI; CA57643; CA; NCI; +

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The phthalocyanines are promising second-generation photosensitizers that are being evaluated for the photodynamic therapy (PDT) of **malignant** tumors. In vivo studies with the silicon phthalocyanine Pc 4 have shown

that it is highly effective at causing regression of RIF-1 tumors in C3H/HeN mice in PDT protocols. Because cutaneous photosensitivity is the major complication of photosensitizers used for PDT, experiments were performed to evaluate the effect of inhibitors of the inflammatory response (cyproheptadine, dexamethasone, pentoxifylline, and tumor necrosis factor alpha [TNF-alpha] antibodies) on Pc 4-induced cutaneous photosensitivity and tumor regression. The C3H/HeN mice were injected with either Pc 4 or Photofrin and were exposed to 86 J/cm² of filtered radiation emitted from a solar simulator. Animals were irradiated at 1, 3, 7, 10, 14 and 28 days postinjection. Cutaneous photosensitivity was assessed using the murine ear-swelling response. Cyproheptadine, dexamethasone, pentoxifylline and TNF-alpha antibodies were administered prior to illumination to assess their ability to block Pc 4-induced cutaneous photosensitivity and to evaluate whether such **treatment** adversely influenced Pc 4 PDT-induced tumor regression. Compared to Photofrin, Pc 4 produced cutaneous photosensitivity that was transient, resolving within 24 h, and that could be elicited for only 10 days after administration. In contrast, Photofrin caused photosensitivity that required 4 days to resolve and could be elicited for at least 1 month after it was administered. The Pc 4-induced cutaneous photosensitivity could be blocked by corticosteroids and an inhibitor of vasoactive amines (cyproheptadine). The TNF-alpha gene **transcription** was found to increase in keratinocytes following **treatment** with Pc 4 and light. The anti-TNF-alpha antibodies and pentoxifylline, an inhibitor of cytokine **transcription**, also prevented cutaneous photosensitivity, implicating TNF-alpha in the pathogenesis of Pc 4-induced cutaneous photosensitivity. None of these agents had any effect on Pc 4 PDT-induced tumor regression. Cyproheptadine, dexamethasone, pentoxifylline and TNF-alpha antibodies may be valuable pharmacologic agents in the management of cutaneous photosensitivity associated with PDT without altering the efficacy of this new therapeutic modality. The findings suggest that it should be possible to devise PDT protocols that block cutaneous photosensitivity without impairing the anti-tumor response to the agents.

18/3,AB/16 (Item 16 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09320628 97228139 PMID: 9091577

Signal transducer and activator of **transcription**-3 (STAT3) is constitutively activated in normal, self-renewing B-1 cells but only inducibly expressed in conventional B lymphocytes.

Karras J G; Wang Z; Huo L; Howard R G; Frank D A; Rothstein T L

Department of Medicine, University Medical Center, Boston, Massachusetts 02118, USA.

Journal of experimental medicine (UNITED STATES) Mar 17 1997,

185 (6) p1035-42, ISSN 0022-1007 Journal Code: 2985109R

Contract/Grant No.: AI129690; AI; NIAID

Comment in J Exp Med. 1997 Mar 17;185(6) 981-4; Comment in PMID 9091589

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Cytokine and growth factor receptor engagement leads to the rapid phosphorylation and activation of latent, cytosolic signal transducers and activators of **transcription** (STAT) proteins, which then translocate to the nucleus where they regulate transcriptional events from specific promoter sequences. STAT3 expression in particular has been associated with Abl, Src, and HTLV-1 transformation of normal cells. B-1 lymphocytes are self-renewing, CD5+ B cells that display a propensity for **malignant** transformation and are the normal counterpart to human chronic lymphocytic leukemias. Further, B-1 cells are characterized by aberrant intracellular signaling, including hyperresponsiveness to phorbol ester PKC agonists. Here we demonstrate that B-1 lymphocytes constitutively express nuclear

activated STAT3, which is not expressed by unmanipulated conventional (B-2) lymphocytes. In contrast, STAT3 activation is induced in B-2 cells after antigen receptor engagement in a delayed fashion (after 3 h). Induction of STAT3 is inhibited by both the serine/threonine protein kinase inhibitor H-7 and the immunosuppressive drug rapamycin and requires de novo protein synthesis, demonstrating novel coupling between sIg and STAT proteins that differs from the classical paradigm for STAT induction by cytokine receptors. The inability of prolonged stimulation of conventional B-2 cells with anti-Ig, a **treatment** sufficient to induce CD5 expression, to result in sustained STAT3 activation suggests that STAT3 is a specific nuclear marker for B-1 cells. Thus, STAT3 may play a role in B cell antigen-specific signaling responses, and its constitutive activation is associated with a normal cell population exhibiting intrinsic proliferative behavior.

18/3,AB/17 (Item 17 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09316001 97233477 PMID: 9078467

Modulatory effects of human cytomegalovirus infection on **malignant** properties of cancer cells.

Cinatl J; Cinatl J; Vogel J U; Rabenau H; Kornhuber B; Doerr H W
Institute of Medical Virology, University Clinics of Frankfurt/Main, Germany.

Intervirolgy (SWITZERLAND) 1996, 39 (4) p259-69, ISSN
0300-5526 Journal Code: 0364265

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Although there is no definitive evidence of the association of human cytomegalovirus (HCMV) infection with human cancers, the oncogenic potential of HCMV has been well established by in vitro studies demonstrating the ability of UV-irradiated or infectious virus to transform a variety of cells. After prolonged passaging the transformed cell type was maintained while HCMV DNA sequences were no more detectable. Three morphological transforming regions (mtr) of HCMV have been identified. The effects of HCMV on cellular functions which may be associated with the **malignant** phenotype include the expression of oncogenes and transcriptional activation of growth **factors** and interleukin synthesis. In infected cells, HCMV induces cytoskeletal alterations and changes in expression of cell surface receptors for extracellular matrix proteins which could result in increased motility and dissemination of cancer cells. Several human neuroblastoma cell lines undergo maturation in different neural crest derived cell types upon **treatment** with oncogenic potential agents, i.e. retinoic acid. The persistent HCMV infection of neuroblastoma cells (> 1 year) is accompanied by the increased expression of oncoproteins (i.e. N-myc) and decreased expression of tyrosine hydroxylase and dopamine-beta-hydroxylase. The activation of the cellular metabolism is due to HCMV binding to cellular receptors (prior to virus gene expression) and to the activity of HCMV immediate early (IE) gene products. IE proteins act directly as transcriptional activators or their activity is mediated by a variety of cellular **transcription factors**. HCMV infection may result in activation of promoters of cellular genes coding for cytokines, replication enzymes, proto-oncogenes and viral promoters. Recently it has been demonstrated that HCMV IE proteins block apoptosis probably by suppressing the ability of the antioncogene p53 to activate a reporter gene. The interactions of HCMV with tumor suppressor proteins such as p53 or retinoblastoma (pRb) susceptibility protein are reminiscent of those mediated by the oncoproteins of DNA tumor viruses. The acquisition of a fully **malignant** phenotype by normal cells is thought to require several mutations in a number of cellular genes. In this connection, HCMV may play

the role of a nonobligate either direct or indirect cofactor for tumor genesis, e.g. by blocking apoptosis, which may be an essential requirement for tumor progression. Due to the stimulation of growth **factors** and/or inhibition of antioncogenes by its gene products, HCMV may modulate the **malignant** potential for tumor cells.

18/3,AB/18 (Item 18 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09294058 97190218 PMID: 9038373

Expression and function of members of the cytokine receptor superfamily on breast cancer cells.

Douglas A M; Goss G A; Sutherland R L; Hilton D J; Berndt M C; Nicola N A ; Begley C G

Rotary Bone Marrow Research Laboratories.

Oncogene (ENGLAND) Feb 13 1997, 14 (6) p661-9, ISSN 0950-9232

Journal Code: 8711562

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Receptors for the cytokines leukemia inhibitory **factor** (LIF), interleukin-6 (IL-6), oncostatin M (OSM), ciliary neurotrophic **factor** (CNTF) and interleukin-11 (IL-11) are members of the structurally conserved hemopoietin receptor superfamily. In addition, they all share the transmembrane signalling protein gp130. In this paper the expression and function of this family of receptors in breast cancer cells was examined. RT-PCR analyses demonstrated that gp130 was expressed in 12/12 breast cell lines and the specific receptor alpha-chains for IL-6, LIF, IL-11 and CNTF were expressed in the majority of these cell lines. This was in contrast to other hemopoietin receptors. Examination of 50 clinical samples of **malignant** breast tissue by RT-PCR showed a similar pattern of expression of gp130 associated receptors. **Treatment** of breast cancer cell lines with OSM resulted in changes in cellular morphology. Cellular proliferation was inhibited following exposure to OSM (3/4 cell lines), IL-11 (2/4 cell lines), and by IL-6 and LIF (1/4 cell lines). Cell surface binding of LIF and OSM was also documented. The expression of these receptors in 12/12 cell lines and greater than 95% of clinical samples suggests that these molecules may be important in regulating the growth of breast cells.

18/3,AB/19 (Item 19 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09280352 97163199 PMID: 9010034

Type I insulin-like growth **factor** receptor gene expression in normal human breast tissue **treated** with oestrogen and progesterone.

Clarke R B; Howell A; Anderson E

Clinical Research Department, Christie Hospital NHS Trust, Withington, Manchester, UK.

British journal of cancer (SCOTLAND) 1997, 75 (2) p251-7,
ISSN 0007-0920 Journal Code: 0370635

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The epithelial proliferation of normal human breast tissue xenografts implanted into athymic nude mice is significantly increased from basal levels by oestradiol (E2), but not progesterone (Pg) **treatment** at serum concentrations similar to those observed in the luteal phase of the human menstrual cycle. Type I IGF receptor (IGFR-I) mRNA and protein have been shown to be up-regulated by E2 in MCF-7 breast cancer cells in vitro

in which IGF-I and E2 act synergistically to stimulate proliferation. We have investigated the expression of the IGFR-I mRNA in normal human breast xenografts **treated** with or without E2 or Pg alone and in combination. Northern analysis of 20 micrograms of RNA extracted from the breast xenograft samples showed no hybridization with 32P-labelled IGFR-I probe, although an 11-kb species of IGFR-I mRNA could be seen when 20 micrograms of RNA extracted from either MCF-7 breast cancer cells or human breast carcinomas was examined in this way. In order to analyse the expression of IGFR-I mRNA in breast xenografts, a quantitative reverse **transcription**-polymerase chain reaction (RT-PCR) was employed in which RNA loading, reverse **transcription** and PCR efficiencies were internally controlled. The data indicate that the IGFR-I mRNA is up-regulated by two to threefold compared with untreated levels by 7 and 14 days **E2 treatment**. In contrast, 7 or 14 days **Pg treatment** down-regulates the receptor mRNA to approximately half that of untreated levels, whereas combination E2 and Pg **treatment** produced a twofold increase in IGFR-I mRNA levels compared with untreated tissue. The results are consistent with the suggestion that E2 may act to stimulate proliferation indirectly via a paracrine mechanism involving IGFs in normal as well as **malignant** human breast epithelial cells.

18/3,AB/20 (Item 20 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09269589 97175150 PMID: 9022810

Regulation of the **transcription factor** AP-1 in benign and **malignant** mouse keratinocyte cells.

Joseloff E; Bowden G T
Department of Radiation Oncology, University of Arizona Health Sciences Center, Tucson 85724, USA.

Molecular carcinogenesis (UNITED STATES) Jan 1997, 18 (1)
p26-36, ISSN 0899-1987 Journal Code: 8811105
Contract/Grant No.: CA23074; CA; NCI; CA40584; CA; NCI; P30ES06694-02; ES
; NIEHS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The mouse benign keratinocyte cell line 308 was previously shown to have less AP-1 DNA binding and transactivation ability than its **malignant** variant 10Gy5. Because elevated AP-1 activity in 10Gy5 appears to be critical for its **malignant** phenotype, we were interested in examining the molecular mechanisms that regulate activator protein 1 (AP-1) in this system. In both 308 and 10Gy5 cells, c-fos, fra-2, c-jun, jun B, and jun D were capable of binding to an AP-1 DNA binding site as determined by antibody clearance gel mobility shift assays. By western analysis, jun B steady-state nuclear and cytoplasmic protein levels were reduced in 10Gy5 cells as compared with 308 cells and jun B steady-state mRNA levels were similar in the two cell lines. The rate of jun B protein synthesis was decreased in 10Gy5 cells in comparison with 308 cells. Gel mobility shift experiments indicated that AP-1 inhibitory proteins were not present in the cytoplasm of 308 cells. Oxidation-reduction posttranslational modification was not a major mechanism of AP-1 regulation in these cells as shown by 12-O-tetradecanoylphorbol-13-acetate-responsive element (TRE) gel mobility shift assay of nuclear protein **treated** with a reducing agent and by western analysis for ref-1 protein. Overall phosphorylation of AP-1 proteins in 308 and 10Gy5 cells was examined by 32P orthophosphate labeling and immunoprecipitation. A difference in jun B protein overall phosphorylation was observed in the two cell lines. Our experiments suggest that decreased jun B protein levels may be a mechanism that results in elevated AP-1 activity in **malignant** 10Gy5 cells.

18/3,AB/21 (Item 21 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09255867 97163529 PMID: 9010349

The effects of dihydrotestosterone on the expression of p185(erbB-2) and c-erbB-2 mRNA in the prostatic cell line LNCaP.

Myers R B; Oelschlager D K; Hockett R D; Rogers M D; Conway-Myers B A; Grizzle W E

Department of Pathology, The University of Alabama at Birmingham, USA.

Journal of steroid biochemistry and molecular biology (ENGLAND) Dec 1996, 59 (5-6) p441-7, ISSN 0960-0760 Journal Code: 9015483

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The c-erbB-2 proto-oncogene encodes a 185000 molecular weight protein (p185(erbB-2)) which shares structural homology with the epidermal growth factor (EGF) receptor. We examined the effects of dihydrotestosterone (DHT) on the expression of p185(erbB-2) and c-erbB-2 mRNA in the human malignant prostatic cell line LNCaP. LNCaP cells grown in steroid-depleted media were treated with DHT (10(-11)-10(-6) M) for 48 h and p185(erbB-2) expression was determined by Western blotting and immunoprecipitation of 35S-methionine labelled p185(erbB-2). c-erbB-2 mRNA levels were determined using a competitive quantitative reverse transcription-polymerase chain reaction (RT-PCR) based technique. DHT at concentrations of 10(-9) M or greater resulted in decreased expression of p185(erbB-2). In contrast, DHT at these levels stimulated EGF receptor protein expression and cellular proliferation. c-erbB-2 mRNA levels declined to 30-50% of control levels following treatment with DHT of 10(-10) M or greater. Furthermore, the inhibitory effects on c-erbB-2 mRNA were rapid, occurring within 6-12 h of treatment. In summary, these results demonstrate that DHT, at concentrations that stimulate cell growth, inhibits the expression of p185(erbB-2) and c-erbB-2 mRNA.

18/3,AB/22 (Item 22 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09231454 97145457 PMID: 8991099

Treatment of melanoma cells with the synthetic retinoid CD437 induces apoptosis via activation of AP-1 in vitro, and causes growth inhibition in xenografts in vivo.

Schadendorf D; Kern M A; Artuc M; Pahl H L; Rosenbach T; Fichtner I; Nurnberg W; Stuting S; von Stebut E; Worm M; Makki A; Jurgovsky K; Kolde G; Henz B M

Department of Dermatology, Virchow Klinikum, Humboldt Universitat zu Berlin, Germany.

Journal of cell biology (UNITED STATES) Dec 1996, 135 (6 Pt 2) p1889-98, ISSN 0021-9525 Journal Code: 0375356

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Human **malignant** melanoma is notoriously resistant to pharmacological modulation. We describe here for the first time that the synthetic retinoid CD437 has a strong dose-dependent antiproliferative effect on human melanoma cells (IC50: 5 x 10(-6) M) via the induction of programmed cell death, as judged by analysis of cell morphology, electron microscopical features, and DNA fragmentation. Programmed cell death was preceded by a strong activation of the AP-1 complex in CD437-treated cells as demonstrated by gel retardation and chloramphenicol transferase (CAT) assays. Northern blot analysis showed a time-dependent increase in the expression of c-fos and c-jun encoding components of AP-1, whereas bcl-2 and p53 mRNA levels remained constant. CD437 also exhibited a strong

growth inhibitory effect on MeWo melanoma cells in a xenograft model. In tissue sections of CD437-**treated** MeWo tumors from these animals, apoptotic melanoma cells and c-fos overexpressing cells were colocalized by TdT-mediated deoxyuridine triphosphate-digoxigenin nick end labeling (TUNEL) staining and in situ hybridization. Taken together, this report identifies CD437 as a retinoid that activates and upregulates the **transcription factor** AP-1, leading eventually to programmed cell death of exposed human melanoma cells in vitro and in vivo. Further studies are needed to evaluate whether synthetic retinoids such as CD437 represent a new class of retinoids, which may open up new ways to a more effective therapy of **malignant** melanoma.

18/3,AB/23 (Item 23 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09199785 97102413 PMID: 8946840

Epstein-Barr virus-driven gene therapy for EBV-related lymphomas.
Franken M; Estabrooks A; Cavacini L; Sherburne B; Wang F; Scadden D T
Division of Hematology/Oncology, Harvard Medical School, Boston,
Massachusetts, USA.

Nature medicine (UNITED STATES) Dec 1996, 2 (12) p1379-82,
ISSN 1078-8956 Journal Code: 9502015
Contract/Grant No.: R01-CA 55520; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Genetic alterations in **malignant** tissues are potential targets for gene-based cancer therapies. Alternatively, aberrant expression of certain specific genes associated with **malignant** transformation may be envisioned to enhance the expression of chemosensitizing drugs. Epstein-Barr virus (EBV)-related B-cell lymphomas are fatal complications of immunosuppression due to AIDS, organ transplantation or congenital immune abnormalities. The **malignant** cells latently infected with EBV typically express the **transcription factor** EBNA2 as one of nine latent viral genes. We tested whether an EBNA2-responsive EBV promoter may selectively target EBV-related lymphoma cells by virus-regulated expression of a suicide gene. Using the BamC promoter driving a hygromycin-thymidine kinase fusion gene or controls, we demonstrated that sensitivity to ganciclovir was selectively enhanced in cells expressing EBNA2. Further, there was complete macroscopic regression of established B-cell lymphomas in mice with severe combined immunodeficiency disease (SCID mice) **treated** with a single course of ganciclovir. These data provide in vitro and in vivo support for a model of exploiting the molecular basis of tumor development to enhance the specificity of gene therapy.

18/3,AB/24 (Item 24 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09184467 97085926 PMID: 8932000

Secondary acute myeloblastic leukemia with t(16;21) (q24;q22). involving the AML1 gene.

Berger R; Le Coniat M; Romana S P; Jonveaux P
INSERM U301, Institut de Genetique Moleculaire, Paris, France.
Hematology and cell therapy (FRANCE) Apr 1996, 38 (2) p183-6,
ISSN 1269-3286 Journal Code: 9613253
Document type: Journal Article

Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A t(16;21) (q24;q22) translocation was detected by fluorescence in situ hybridization in a patient with acute myeloblastic leukemia previously

treated for **malignant** lymphoma. While the breakpoint on chromosome 21 was within the AML1 gene as determined by FISH, the gene partner on chromosome 16 could not be identified. Band 16q24 appears to be rearranged in several types of myeloid proliferation and a review of the literature shows that these rearrangements most often occur in secondary leukemia and myelodysplastic syndrome or are part of complex chromosomal rearrangements.

18/3,AB/25 (Item 25 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09172723 97079185 PMID: 8920917

A cis-trans interaction at the 3'-untranslated region of ribonucleotide reductase mRNA is regulated by TGF-beta 1, TGF-beta 2, and TGF-beta 3.

Amara F M; Smith G M; Kuschak T I; Takeuchi T L; Wright J A
Manitoba Institute of Cell Biology, University of Manitoba, Winnipeg, Canada.

Biochemical and biophysical research communications (UNITED STATES) Nov 12 1996, 228 (2) p347-51, ISSN 0006-291X Journal Code: 0372516

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The R2 component of ribonucleotide reductase is rate-limiting for DNA synthesis in proliferating cells, and recently, it has been shown that aberrant expression of R2 directly alters the **malignant** potential of tumor cells. We show that R2 gene expression is elevated in BALB/c 3T3 cells **treated** with transforming growth **factor** (TGF)-beta 1, TGF-beta 2, or TGF-beta 3, as determined by Northern blot analysis. Gel shift assays and UV crosslinking studies demonstrated similar post-transcriptional regulation at the 3'-untranslated region (3'-UTR) of the R2 mRNA, by TGF-beta 1, TGF-beta 2, and TGF-beta 3. The three growth **factors** induced a common 75 kDa RNA-protein complex. A 9 nucleotide sequence, GAGUUUGAG, previously shown to be responsive to TGF-beta 1-mediated R2 message stability changes, effectively competed out the formation of the R2 3'-UTR complex. We propose that these three different members of the TGF-beta family work through a common mechanism to control an important component of cell proliferation and a potential determinant of **malignant** progression.

18/3,AB/26 (Item 26 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09172075 97075136 PMID: 8917562

Induction of apoptosis in rhabdomyosarcoma cells through down-regulation of PAX proteins.

Bernasconi M; Remppis A; Fredericks W J; Rauscher F J; Schafer B W

Department of Pediatrics, University of Zurich, Switzerland.

Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Nov 12 1996, 93 (23) p13164-9, ISSN

0027-8424 Journal Code: 7505876

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The expression of a number of human paired box-containing (PAX) genes has been correlated with various types of tumors. Novel fusion genes encoding chimeric fusion proteins have been found in the pediatric **malignant** tumor alveolar rhabdomyosarcoma (RMS). They are generated by two chromosomal translocations t(2;13) and t(1;13) juxtaposing PAX3 or PAX7, respectively, with a forkhead domain gene FKHR. Here we describe that specific down-regulation of the t(2;13) translocation product in alveolar

RMS cells by antisense oligonucleotides results in reduced cellular viability. Cells of embryonal RMS, the other major histiotype of this tumor, were found to express either wild type PAX3 or PAX7 at elevated levels when compared with primary human myoblasts. **Treatment** of corresponding embryonal RMS cells with antisense oligonucleotides directed against the mRNA translational start site of either one of these two **transcription factors** similarly triggers cell death, which is most likely due to induction of apoptosis. Retroviral mediated ectopic expression of mouse Pax3 in a PAX7 expressing embryonal RMS cell line could partially rescue antisense induced apoptosis. These data suggest that the PAX3/FKHR fusion gene and wild-type PAX genes play a causative role in the formation of RMS and presumably other tumor types, possibly by suppressing the apoptotic program that would normally eliminate these cells.

18/3,AB/27 (Item 27 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09138323 97054164 PMID: 8898543

Perturbing gene expression with oligodeoxynucleotides: research and potential therapeutic applications.

Gewirtz A M

University of Pennsylvania School of Medicine, Stellar-Chance Laboratories, Philadelphia 19104, USA.

Mount Sinai journal of medicine, New York (UNITED STATES) Oct-Nov 1996, 63 (5-6) p372-80, ISSN 0027-2507 Journal Code: 0241032

Contract/Grant No.: CA 66731; CA; NCI; PO1 CA 51083; CA; NCI; R01 CA 54384; CA; NCI

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The ability to block gene function with antisense oligodeoxynucleotides has become an important tool in many research laboratories. Because activation and aberrant expression of proto-oncogenes seems to be an important mechanism in **malignant** transformation, targeted disruption of these genes and other molecular targets with oligodeoxynucleotides could have significant therapeutic utility as well. In this regard, the potential therapeutic usefulness of oligodeoxynucleotides has been demonstrated in many systems and against several different targets including viruses, oncogenes, proto-oncogenes and an increasing array of cellular genes. These studies in aggregate suggest that synthetic oligomers have the potential to become an important new therapeutic agent for the **treatment** of cancer in humans. Nevertheless, it is clear that considerable optimization will be required before antisense oligonucleotides will emerge as an effective agent for **treating** disease in humans. Progress will need to occur on several fronts. Included are issues related to the chemistry of the molecules used: for example, how chemical modification has an impact on uptake, stability, and hybridization efficiency of the synthetic DNA molecule. A clearer understanding of the mechanism of antisense-mediated inhibition, including where such inhibition takes place, will also be required. Finally, cellular "defense" mechanisms, such as increasing **transcription** of the targeted message, may also be **factors** to consider in planning effective **treatment** strategies with these agents. Finally, choice of target is also obviously an important issue.

18/3,AB/28 (Item 28 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09127429 97008860 PMID: 8855977

Dysregulation of autocrine TGF-beta isoform production and ligand responses in human tumour-derived and Ha-ras-transfected keratinocytes and fibroblasts.

Fahey M S; Paterson I C; Stone A; Collier A J; Heung Y L; Davies M; Patel V; Parkinson E K; Prime S S

Department of Oral and Dental Science, University of Bristol, UK.
British journal of cancer (SCOTLAND) Oct 1996, 74 (7) p1074-80

, ISSN 0007-0920 Journal Code: 0370635

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

This study examined the autocrine production of TGF-beta 1, -beta 2 and -beta 3 in culture supernatants from tumour-derived (H series, n = 7; BICR series, n = 5), Ha-ras-transfected (n = 4) and normal (n = 2) human keratinocytes using a sandwich enzyme-linked immunosorbent assay (ELISA). Detection limits were 39.0 pg ml⁻¹ for TGF-beta 1, 78.0 pg ml⁻¹ for TGF-beta 2 and 1.9 ng ml⁻¹ for TGF-beta 3. Tumour-derived oral keratinocytes predominantly produced less TGF-beta 1 than normal oral epithelial cells; the expression of endogenous TGF-beta 2 was variable. In keratinocytes containing mutant Ha-ras, TGF-beta 1 production was enhanced and TGF-beta 2 was undetectable. TGF-beta 3 mRNA was detected by reverse transcription-polymerase chain reaction (RT-PCR) but the protein was not detected in conditioned media, most probably because of the low detection limits of the ELISA for this isoform. Neutralisation experiments indicated that the latent TGF-beta peptide was secreted in keratinocyte conditioned medium. Seven tumour-derived keratinocyte cell lines (H series) and fibroblasts separated from normal (n = 1) and tumour-derived (n = 2) keratinocyte cultures were examined for their response to exogenous TGF-beta 1, -beta 2 and -beta 3. Six of seven tumour-derived keratinocyte cell lines were inhibited by TGF-beta 1 and TGF-beta 2 (-beta 1 > -beta 2); one cell line was refractory to both TGF-beta 1 and TGF-beta 2. Keratinocytes were inhibited (4 of 7), stimulated (1 of 7) or failed to respond (2 of 7) to TGF-beta 3, TGF-beta 1, -beta 2 and -beta 3 stimulated both normal and tumour-associated fibroblasts, but the tumour-associated fibroblasts showed less response to the ligands than their normal counterparts following prolonged treatment with each isoform. The results demonstrate variable autocrine production of TGF-beta isoforms by malignant keratinocytes, with loss of TGF-beta 1 generally associated with the tumour-derived phenotype and modification of endogenous isoform production dependent on the genetic background of the tumour cells. Further, the variable response of the tumour-derived keratinocytes and contiguous fibroblasts to the TGF-beta isoforms suggests that dysregulation of TGF-beta autocrine and paracrine networks are common characteristics of squamous epithelial malignancy.

18/3,AB/29 (Item 29 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09122423 97023576 PMID: 8869935

Curable and non-curable malignancies: lessons from paediatric cancer.
Toren A; Amariglio N; Rechavi G

Pediatric Hemato-Oncology Unit, Chaim Sheba Medical Center, Tel-Hashomer, Israel.

Medical oncology (Northwood, London, England) (ENGLAND) Mar 1996,
13 (1) p15-21, ISSN 1357-0560 Journal Code: 9435512

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The tremendous progress achieved in understanding the molecular basis of cancer, was unfortunately not followed by a mutual improvement in the morbidity and mortality of adult cancer. In contrast, the success rate achieved in paediatric oncology has increased significantly during the past 30 years, and more than two-thirds of the children with cancer can now be cured. p53 has been shown to have a central role on apoptosis in various

cells. As apoptosis is a final common pathway for much of our anti cancer therapy, resistance to apoptosis due to a normal activity of p53 is an important mechanism of tumor resistance and **treatment** failure. Contrary to the findings in most adult tumors, where about 50% of the tumors lack p53 activity, the rate of p53 mutations in childhood cancer is surprisingly low. This may be the key to the much better prognosis of children with cancer. In most adult tumors, multiple genetic events, between five and seven, are usually involved. The oncogenes involved in such tumors usually represent those located upstream of the nuclear **transcription factors**. In most paediatric tumors, in contrast, the initiating event is the activation of nuclear **transcription factors** secondary to chromosomal translocations. It can be speculated that multiple events activating various components of the signal transduction machinery are needed for tumorigenesis, and hence the evolution and progression of such tumors is slow. Moreover, if the **malignant** cell has to accumulate multiple mutations, the chances of crippling the apoptotic mechanism are higher. Genomic instability evidenced by microsatellite variation has been found in colon, pancreas, breast, liver and ovarian adult tumors, and not in paediatric tumors. As multiple somatic mutations are needed for the initiation and progression of the common adult malignancies, inherent genomic instability can dispose to accumulation of multiple mutations. All these molecular interactions are discussed with relevance to the difference between non-curable, mostly adult tumors, and curable, mostly paediatric tumors.

18/3,AB/30 (Item 30 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09046120 96413683 PMID: 8816837

Expression of retinoic acid receptors in squamous cell carcinomas and their possible implication for chemoprevention.

Issing W J; Wustrow T P

Klinik fur Hals-Nasen-Ohrenkranke der Ludwig-Maximilians-Universitat, Klinikum Grosshadern, Munchen, Germany.

Anticancer research (GREECE) Jul-Aug 1996, 16 (4C) p2373-7,
ISSN 0250-7005 Journal Code: 8102988

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Retinoic acid and its natural and synthetic analogs (retinoids) affect a wide array of biological processes. Retinoids are used in the **treatment** of many skin diseases and are promising drugs for several cancers. Most of their actions are thought to result from changes in gene expression which is caused via nuclear retinoic acid receptors and retinoid X receptors. We conducted a study to determine whether the expression of these receptors is different in **malignant** tumors and tumor cell lines versus normal tissue. We performed reverse **transcription** PCR from 29 tissue specimens of squamous cell carcinomas and one melanoma of the head and neck, as well as from 13 cell lines established from head and neck cancer. We were looking for the expression pattern of RAR alpha, beta, gamma and RXR alpha. Only RAR gamma was expressed 100% in cell lines and tissue specimens. RAR beta showed 100% expression in tissue specimens whereas 54% expression in cell lines was seen. All other receptors were diminished in their expression. In the positive controls all receptors were expressed at 100%. The expression of RAR alpha and RAR beta was partially lost in cell lines established from squamous cell carcinoma of the head and neck. The 100% expression of RAR beta in tissue samples versus 54% in cell lines can be explained by the clonal growth of **malignant** cells in cell lines and also possible "contamination" by normal cells in the tissue specimen. In concordance with the literature it seems that RAR alpha and beta play a pivotal role in mediating the response to retinoids.

18/3,AB/31 (Item 31 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09034057 96399633 PMID: 8806104

Regulation of nuclear oncogenes expressed in lung cancer cell lines.

Sabichi A L; Birrer M J

Biomarkers and Prevention Research Branch, National Cancer Institute,
Rockville, Maryland 20850, USA.

Journal of cellular biochemistry. Supplement (UNITED STATES) 1996

, 24 p218-27, ISSN 0733-1959 Journal Code: 8207539

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Lung cancer is a major cause of mortality in the United States and accounts for the majority of all cancer deaths in both men and women. It is hoped that through broadening our understanding of the mechanisms involved in transformation of bronchial epithelial cells we will be able to improve methods of diagnosis and **treatment** of this disease, with the ultimate goal of reducing on lung cancer mortality. A knowledge of the molecular mechanisms involved in processes such as cell division and differentiation is paramount to this task, because it is known that aberrant responses to growth **factors** or cytokines found in the normal cellular milieu can lead to abnormal cell growth and/or transformation. Signals initiated at the cell membrane by tumor promoters, growth **factors**, or cytokines are transduced from the cell membrane to the nucleus and are, in part, mediated centrally by **transcription factors** encoded by nuclear protooncogenes. The **transcription factors** myc, jun, and fos have been characterized in both normal and transformed lung epithelial cells through detailed studies using cell lines. In this manuscript, we review what is known about the expression and regulation of these nuclear protooncogenes in normal and **malignant** epithelial cells of the lung, and their role in the development of lung cancer.

18/3,AB/32 (Item 32 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08985083 96351971 PMID: 8750626

Interleukin-1 and tumor necrosis **factor** alpha induce class 1 aldehyde dehydrogenase mRNA and protein in bone marrow cells.

Moreb J S; Turner C; Sreerama L; Zucali J R; Sladek N E; Schweder M
Department of Medicine, University of Florida, Gainesville 32610-0277,
USA.

Leukemia & lymphoma (SWITZERLAND) Dec 1995, 20 (1-2) p77-84,
ISSN 1042-8194 Journal Code: 9007422

Contract/Grant No.: AI24709; AI; NIAID; CA21737; CA; NCI; R29-CA59684; CA
; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Interleukin-1 (IL-1) and tumor necrosis **factor** alpha (TNF alpha) protect normal human hematopoietic progenitors from the toxicity of 4-hydroperoxycyclophosphamide (4-HC). Aldehyde dehydrogenase Class 1 (ALDH-1) is the enzyme that inactivates 4-HC. Diethylaminobenzaldehyde (DEAB), a competitive inhibitor of ALDH-1, was shown to prevent the protective effects of IL-1 and TNF alpha. In this study, we examined the effect of IL-1 and TNF alpha on the expression of ALDH-1 in normal bone marrow as well as **malignant** cells. ALDH-1 mRNA and protein were quantified using Northern and Western blotting, respectively. In addition, the ALDH-1 enzyme activity in untreated as well as IL-1 and TNF alpha **treated** bone marrow cells was determined spectrophotometrically. The

role of glutathione (GSH) in the protection against 4-HC toxicity was also studied. The results show that pretreatment with IL-1 and TNF alpha for 6 h or 20 h increase the expression of ALDH-1 mRNA and protein, respectively, in human bone marrow cells. In contrast, IL-1 and TNF alpha **treatment** did not affect the ALDH-1 expression in several leukemic and solid tumor cell lines, regardless of whether or not ALDH-1 is expressed constitutively. Furthermore, the ALDH-1 enzyme activity was significantly induced in bone marrow cells after 20 h pre-**treatment** with IL-1 and TNF alpha. Finally, the depletion of or inactivation of GSH did not affect the protection against 4-HC toxicity. In conclusion, inhibition of the protection from 4-HC toxicity by DEAB, together with the increase in ALDH-1 expression and activity, provide strong evidence that IL-1 and TNF alpha mediate their protective action, at least partially, through ALDH-1.

18/3,AB/33 (Item 33 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08966563 96330347 PMID: 8760309

Abnormalities of p16, p15 and CDK4 genes in recurrent **malignant** astrocytomas.

Saxena A; Robertson J T; Ali I U

Surgical Neurology Branch, National Institute of Neurological Disorder and Stroke, NIH, Bethesda, Maryland, USA.

Oncogene (ENGLAND) Aug 1 1996, 13 (3) p661-4, ISSN 0950-9232

Journal Code: 8711562

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Abnormalities in the p16, p15 and CDK4 genes that regulate transition through the G1 phase of the cell cycle have been implicated in the **malignant** progression of astrocytomas. The results of the present study demonstrate that dysfunction of these genes also occurs during recurrence of glial tumors that were highly **malignant** at first presentation. Analysis of 10 matched pairs of high grade **malignant** astrocytomas and their subsequent recurrences identified three distinct groups. The primary and recurrent tumors in Group A did not show structural alterations in the p16, p15 or CDK4 genes, whereas homozygous codeletion of p16 and p15 was observed in both primary and recurrent tumors in Group B. The primary tumors in Group C had a normal profile of p16, p15 and CDK4 at presentation. Upon recurrence, however, the tumors sustained either deletion of p16 alone or codeletion of both p16 and p15 or amplification of CDK4. Analysis of the molecular differences between primary anaplastic astrocytomas/glioblastomas and their subsequent recurrences, which are clinically indistinguishable, may provide better therapeutic options for **treatment**.

18/3,AB/34 (Item 34 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08934238 96292236 PMID: 8700540

Oxamflatin: a novel compound which reverses **malignant** phenotype to normal one via induction of JunD.

Sonoda H; Nishida K; Yoshioka T; Ohtani M; Sugita K

Shionogi Research Laboratories, Shionogi & Co., Ltd., Osaka, Japan.

Oncogene (ENGLAND) Jul 4 1996, 13 (1) p143-9, ISSN 0950-9232

Journal Code: 8711562

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

In the course of screening for inhibitors of tumorigenic phenotype of

K-ras-transformed NIH3T3 cells (DT cells), we found a novel compound, oxamflatin, an aromatic sulfonamide hydroxamate derivative, which induces flat phenotype in these cells and suppresses their anchorage-independent growth. In contrast to DT cells, in v-raf-transformed NIH3T3 cells, no change in their morphology and no specific inhibition of their anchorage-independent growth was observed. Interestingly, oxamflatin was effective to NIH3T3 cells transformed by constitutively activated mutant of MEK, indicating the possibility that oncogene-induced morphological change is not necessarily induced by common signaling pathway such as MAP kinase cascade. In oxamflatin-treated DT cells, the expression of **transcription factor** junD was highly augmented, resulting in trans-activation of fibronectin gene by junD via cyclic AMP responsive element in its promoter. This behavior of junD was confirmed to correlate well with partial blocking of **malignant** phenotype in DT cells. Thus, oxamflatin can be categorized as the first reagent which induces genes whose products can interfere with oncogene-dependent transformation.

18/3,AB/35 (Item 35 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08929185 96283578 PMID: 8683990

Expression of the LH2 gene in chronic myeloid leukaemia cells.

Al-Jehani F; Hochhaus A; Spencer A; Goldman J M; Melo J V

LRF Centre for Adult Leukaemia, Royal Postgraduate Medical School, Hammersmith Hospital, London, UK.

Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K (ENGLAND) Jul 1996, 10 (7) p1122-6, ISSN

0887-6924 Journal Code: 8704895

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The LH2 gene encodes a putative **transcription factor** containing two N-terminal LIM and one C-terminal HOX domains. The LH2 locus was mapped to 9q33-34.1, centromeric to the ABL gene. In a recent report, it was suggested that high levels of LH2 expression are consistently observed in chronic myeloid leukemia (CML) patients, whereas no **transcription** is detected in normal individuals. This led to the hypothesis that aberrant expression of LH2 may represent an additional mechanism for **malignant** cell proliferation in CML. We have studied the expression of LH2 in leucocytes from patients with CML or with other chronic myeloproliferative disorders (CMD), and from normal individuals, using an optimised reverse-**transcription** and polymerase chain reaction (PCR) technique. Twenty-seven out of 29 cDNA samples from normal individuals (93%), 49 out of 51 samples from CML patients (96%) and 20 out of 20 from Philadelphia chromosome-negative CMD showed evidence of LH2 expression. Similarly, LH2 **transcription** was also detected in leucocytes from CML patients in complete cytogenetic remission after **treatment** with interferon-alpha. Furthermore, all 36 EBV-induced lymphoblastoid cell lines established from six chronic phase CML patients showed unequivocal LH2 expression, regardless of the BCR-ABL status of the line (9 BCR-ABL positive, 27 BCR-ABL negative). We conclude that LH2 expression is not confined to CML cells, and that the t(9;22)(q34;q11) does not promote 'de novo' transcriptional activation of this gene.

18/3,AB/36 (Item 36 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08911954 96252279 PMID: 8707896

Basic fibroblast growth **factor** selectively regulates ornithine decarboxylase gene expression in **malignant** H-ras transformed cells.

Hurta R A; Huang A; Wright J A

Manitoba Institute of Cell Biology, University of Manitoba, Winnipeg, Canada.

Journal of cellular biochemistry (UNITED STATES) Mar 15 1996, 60
(4) p572-83, ISSN 0730-2312 Journal Code: 8205768

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Cell growth regulation by fibroblast growth factors (FGFs) is highly complex. The present study demonstrates a novel link between alterations in bFGF regulation during **malignant** conversion and the expression of ornithine decarboxylase, a key rate-limiting and regulatory activity in the biosynthesis of polyamines. H-ras transformed mouse 10T 1/2 cell lines exhibiting increasing **malignant** potential were investigated for possible bFGF-mediated changes in ornithine decarboxylase gene expression. Selective induction of ornithine decarboxylase gene expression was observed, since, in contrast to nontransformed 10T 1/2 cells and cells capable of only benign tumor formation, H-ras transformed metastatic cells exhibited marked elevations in ornithine decarboxylase message levels. Evidence for regulation of ornithine decarboxylase gene expression by bFGF at both **transcription** and posttranscription was found. Actinomycin D pretreatment of **malignant** cells prior to bFGF exposure inhibited the increase in ornithine decarboxylase message. Furthermore, striking differences in the rates of ornithine decarboxylase message decay were observed when cells **treated** with bFGF were compared to untreated control cells, with the half-life of ornithine decarboxylase mRNA increasing from 2.4 h in untreated cells to 12.5 h in cells exposed to bFGF. Evidence was also obtained for a cycloheximide-sensitive regulator of ornithine decarboxylase gene expression whose effect, in combination with bFGF, resulted in a further augmentation of ornithine decarboxylase gene expression. Furthermore, evidence is presented to suggest a possible role for G-protein-coupled events in the bFGF-mediated regulation of ornithine decarboxylase gene expression. The bFGF regulation of ornithine decarboxylase expression in H-ras transformed **malignant** cells appeared to occur independent of protein kinase C-mediated events. These results show that bFGF can modulate ornithine decarboxylase gene expression in **malignant** H-ras transformed cells and further suggests a mechanism of growth factor stimulation of **malignant** cells wherein early alterations in the regulatory control of ornithine decarboxylase gene expression are critical.

18/3,AB/37 (Item 37 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08893509 96258428 PMID: 8777434

Suppression of oncogene-induced transformation by quercetin and retinoic acid in rat liver epithelial cells.

Lagarigue S; Chaumontet C; Heberden C; Martel P; Gaillard-Sanchez I
Laboratoire de Nutrition et Securite Alimentaire, INRA, Jouy-en-Josas, France.

Cellular & molecular biology research (UNITED STATES) 1995, 41
(6) p551-60, ISSN 0968-8773 Journal Code: 9316986

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

AP1 is a heterodimeric complex containing products of the Jun and Fos oncogene families. The c-fos and c-jun protooncogenes act as transcriptional activator for numerous cellular genes, and the overexpression of these genes may cause **malignant** transformation. In this study, to show evidence of a possible inhibition of AP1 transcriptional activity in molecular mechanisms of foodborne molecules, known to be negative modulators of carcinogenesis, we established two rat

liver epithelial (REL) cell lines overexpressing either c-fos (43C line) or c-jun (RELCJ1 line) oncoproteins. Contrary to the 43C line, which was spontaneously transformed, the c-jun-transfected REL cells were only transformed in vitro after 12-O-tetra-decanoylphorbol 13-acetate (TPA) exposure. All trans-retinoic acid (RA) abolished the transformation of the 43C line and TPA-treated RELCJ1 cells, suggesting that RA could decrease AP1 activity in these cells despite c-fos or c-jun overexpression. Furthermore, we show for the first time that a flavonoid, quercetin, which is a natural component of vegetables, inhibited only the transformation of the 43C line. The spontaneous transformation of the c-fos-transfected REL cells was associated with the appearance of c-fos/AP1 complexes binding TRE, suggesting that c-fos/AP1 complexes are involved in the antitransforming mechanism of quercetin.

18/3,AB/38 (Item 38 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08891805 96249454 PMID: 8664375

Activation of the radiosensitive EGR-1 promoter induces expression of the herpes simplex virus thymidine kinase gene and sensitivity of human glioma cells to ganciclovir.

Joki T; Nakamura M; Ohno T

Department of Neurosurgery, The Joki University of School of Medicine, Tokyo, Japan.

Human gene therapy (UNITED STATES) Dec 1995, 6 (12) p1507-13,
ISSN 1043-0342 Journal Code: 9008950

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Herein we describe experiments showing that the early growth response gene 1 (EGR-1) promoter is sufficient to confer selective expression of the luciferase gene (Luc) in glioma cell lines exposed to ionizing radiation. Activity of the EGR-1 promoter was investigated in human glioblastoma cells using the plasmid vector, pEGR-Luc. The EGR-1 promoter gene directed radiosensitive expression of luciferase. This promoter showed high levels of activity (10-fold) in irradiated glioma cell lines as compared to basal levels of activity in nonirradiated cell lines. Maximum activation was detectable at 1-3 hr after stimulation with 20 Gy. The results also demonstrate that cells modified to contain the herpes simplex virus-thymidine kinase (HSV-tk) gene under control of the EGR-1 promoter become sensitive to **treatment** with the antiviral agent ganciclovir (GCV), whereas nonirradiated cells and nontransfected cells were unaffected by this agent. This results suggest that therapeutic genes can be expressed selectively in irradiated glioma cells. The results also indicate that the EGR-1 promoter can be used to induce exogenous genes selectively in radiation fields used for the **treatment of malignant brain tumors**.

18/3,AB/39 (Item 39 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08825638 96180988 PMID: 8605252

Regulation of differential expression of platelet-derived growth factor alpha- and beta-receptor mRNA in normal and **malignant** human mesothelial cell lines.

Langerak A W; van der Linden-van Beurden C A; Versnel M A

Department of Immunology, Erasmus University, Rotterdam, The Netherlands.

Biochimica et biophysica acta (NETHERLANDS) Feb 7 1996, 1305
(1-2) p63-70, ISSN 0006-3002 Journal Code: 0217513

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

In earlier studies we showed that the expression of patterns of platelet-derived growth factor (PDGF) alpha- and beta-receptors differ between normal and **malignant** mesothelial cell lines. Normal mesothelial cells predominantly express PDGF alpha-receptor mRNA and protein, whereas most **malignant** mesothelioma cell lines produce PDGF beta-receptor mRNA and protein. In this paper we studied regulation of this differential PDGF receptor mRNA expression. Such an analysis is of importance in view of the suggested PDGF autocrine activity involving the PDGF beta-receptor mesothelioma cells. The results obtained in this study demonstrate that **malignant** mesothelioma cell lines are not only capable of PDGF beta-receptor **transcription** but of alpha-receptor **transcription** as well, as evidenced from run off analysis and RT-PCR using alpha-receptor specific primers. However, the fact that PDGF alpha-receptor mRNA could not be detected by Northern blot analysis, even after cycloheximide **treatment**, suggests a difference in steady-state PDGF alpha-receptor mRNA expression levels between normal and **malignant** mesothelial cell lines, which is likely to be caused by a post-transcriptional mechanism. In normal mesothelial cells a half-life of more than 6 h was observed for PDGF alpha-receptor mRNA. In the majority of **malignant** mesothelioma cell lines clear PDGF beta-receptor mRNA expression was seen. The half-life of the PDGF beta-receptor transcript was at least 6 h in these cells. In contrast, hardly any PDGF beta-receptor **transcription** was observed in run off assays in normal mesothelial cells, suggesting that differences in beta-receptor transcriptional initiation most probably account for the inability to clearly detect PDGF beta-receptor transcripts in these cells. Transforming growth factor beta-1 (TGF-beta 1), which is being produced in active form by mesothelial cells was evaluated for its potential role in regulation of the differential PDGF receptor expression in these cells. Stimulation with TGF-beta 1 revealed decreased PDGF alpha-receptor mRNA expression in normal mesothelial cells. The effect on PDGF beta-receptor mRNA in the **malignant** mesothelioma cell lines was variable. Although the TGF-beta 1 effect cannot entirely explain the differential PDGF receptor expression pattern, TGF-beta 1 may nevertheless play a role in downregulation of an (already) low PDGF alpha-receptor mRNA level in **malignant** mesothelioma cell lines.

18/3,AB/40 (Item 40 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08820065 96152096 PMID: 8592143

Lipid metabolism as a target for brain cancer therapy: synergistic activity of lovastatin and sodium phenylacetate against human glioma cells.

Prasanna P; Thibault A; Liu L; Samid D

Clinical Pharmacology Branch, National Cancer Institute, Bethesda, Maryland, USA.

Journal of neurochemistry (UNITED STATES) Feb 1996, 66 (2)
p710-6, ISSN 0022-3042 Journal Code: 2985190R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Malignant gliomas, the most common form of primary brain tumors, are highly dependent on the mevalonate (MVA) pathway for the synthesis of lipid moieties critical to cell replication. Human glioblastoma cells were found to be uniquely vulnerable to growth arrest by lovastatin, a competitive inhibitor of the enzyme regulating MVA synthesis, 3-hydroxy-3-methylglutaryl coenzyme A reductase. The sodium salt of phenylacetic acid (NaPA), an inhibitor of MVA-pyrophosphate decarboxylase, the enzyme that controls MVA use, acted synergistically with lovastatin to suppress **malignant** growth. When used at pharmacologically attainable

concentrations, the two compounds induced profound cytostasis and loss of **malignant** properties such as invasiveness and expression of the transforming growth factor -beta 2 gene, coding for a potent immunosuppressive cytokine. Supplementation with exogenous ubiquinone, an end product of the MVA pathway, failed to rescue the cells, suggesting that decreased synthesis of intermediary products are responsible for the antitumor effects observed. In addition to blocking the MVA pathway, lovastatin alone and in combination with NaPA increased the expression of the peroxisome proliferator-activated receptor, a **transcription factor** implicated in the control of lipid metabolism, cell growth, and differentiation. Our results indicate that targeting lipid metabolism with lovastatin, used alone or in combination with the aromatic fatty acid NaPA, may offer a novel approach to the **treatment of malignant gliomas**.

18/3,AB/41 (Item 41 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08791816 96141136 PMID: 8590836

Detection of chromosomal translocations in leukemia-lymphoma cells by polymerase chain reaction.

Drexler H G; Borkhardt A; Janssen J W

DSM-German Collection of Microorganisms and Cell Cultures, Department of Human and Animal Cell Cultures, Braunschweig, Germany.

Leukemia & lymphoma (SWITZERLAND) Nov 1995, 19 (5-6) p359-80,
ISSN 1042-8194 Journal Code: 9007422

Document type: Journal Article; Review; Review, Academic
Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

In recent years many chromosomal translocations involved in leukemia and lymphoma have been defined at the molecular level. In addition to advancing the understanding of pathological mechanisms underlying the transformation process, the cloning and sequencing of the genes altered by the translocations have provided new tools for diagnosis and monitoring of patients. In particular, the polymerase chain reaction (PCR) methodology yields rapid, sensitive and accurate diagnostic and prognostic information. As leukemias carrying certain translocations confer a higher risk of **treatment** failure, it is important to identify accurately all positive cases in order to give appropriate therapy. An important new initiative in the diagnostic setting and anti-leukemic therapy is the early detection of minimal residual disease (MRD). If MRD, implying an increased risk of relapse, is reliably detected during apparent clinical remission, alternative strategies could be applied early while the **malignant** cell burden is still minimal. The PCR assays are clearly more sensitive than other methods of MRD detection including morphology, immunophenotyping and cytogenetics; **treatment** failure is first detectable by PCR followed by cytogenetic relapse and finally clinical disease. PCR assays have been most often used in the MRD analysis of follicular lymphoma with t(14;18), chronic myeloid leukemia and acute lymphoblastic leukemia (ALL) with t(9;22), ALL with t(4;11), and acute myeloid leukemia (AML) with t(8;21) or t(15;17). PCR amplification is applicable to any other translocation provided the translocation is highly associated with the malignancy and the breakpoints are sufficiently clustered; a quickly increasing number of such specific molecular markers are now available for PCR assays. PCR still remains an experimental investigation for the detection of covert disease. However, the clinical relevance of MRD detection should be evaluated separately for each type of leukemia as significant prognostic differences between disease entities were found. This review describes the PCR assays available for the detection of leukemia cells with specific chromosomal translocations and summarizes the experience with the application of PCR techniques in monitoring patients during the course of the disease.

18/3,AB/42 (Item 42 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08786386 96147982 PMID: 8571707

[An EGFR antisense oligodeoxynucleotides and its phosphorothioate analogue inhibit the growth of human hepatocarcinoma BEL-7404 cells]

Lin J Q; Xu Z S; Zhang H Y; Yang Z W; Xu Y H

Shanghai Institute of Cell Biology, Academia Sinica.

Shi yan sheng wu xue bao = Journal of experimental biology (CHINA) Sep 1995, 28 (3) p241-6, ISSN 0001-5334 Journal Code: 0413570

Document type: Journal Article ; English Abstract

Languages: CHINESE

Main Citation Owner: NLM

Record type: Completed

We have previously reported that the **malignant** phenotype of human liver carcinoma cell line BEL-7404 was reversed by antisense EGFR RNA. The aim of this paper is to explore the effects of an oligomer targeted to mRNA for EGFR and growth of BEL-7404 cells. A 21-mer oligodeoxynucleotides (ODNs) complementary to the 5' initiation region of mRNA for EGFR was synthesized and added to medium. The results showed that the growth of BEL-7404 cells was inhibited by ODNs at concentration of 3.2 mumol/L. Inhibition of DNA synthesis of BEL-7404 cells was dose-dependent and reached to 62.1% at 3.2 mumol/L as measured by 3H-thymidine incorporation test. The inhibition of EGFR gene **transcription** of the cells was up to 10.5% and 14.3% respectively after incubation with ODNs by 5 and 24 hours as measured by densitometric scanning of dot (RNA) blots of EGFR. The EGFR protein (P 170) expression was also found to be blocked by 4 days' antisense oligomer **treatment** up to 37.4% as measured by densitometric scanning of specific band of Western blot. The oligonucleotide phosphorothioate (S-ODNs) complementary to the same region of the gene was also synthesized and its growth inhibition effects on BEL-7404 cells were compared with those of unmodified oligomers. ODNs attained their highest effect within 30 hours. The proliferation inhibition rate of the cells didn't increase when cells were cultured in serum free medium. In contrast, the S-ODNs induced inhibition reached comparable level after 96 hours **treatment** as measured by 3H-thymidine uptake and the effect lasted longer, 1 mumol/L S-ODNs showed a little effect on BEL-7404 cells' proliferation. We concluded that the antisense oligomers directed to mRNA for EGFR could inhibit the BEL-7404 cells growth by blocking the EGFR gene expression in some degree and the phosphorothioate analogues were more stable than the unmodified ODNs in vitro.

18/3,AB/43 (Item 43 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08754569 96116915 PMID: 8535226

Antiproliferative effect of human interleukin-4 in human cancer cell lines: studies on the mechanism.

Topp M S; Papadimitriou C A; Eitelbach F; Oelmann E; Koehler B; Oberberg D; Von Marschall Z; Reufi B; Stein H; Thiel E; et al

Department of Hematology and Oncology, Benjamin Franklin Hospital (Klinikum Steglitz), Freie Universitaet Berlin, Germany.

Leukemia & lymphoma (SWITZERLAND) Oct 1995, 19 (3-4) p319-28, ISSN 1042-8194 Journal Code: 9007422

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Interleukin-4 (IL-4) plays an important role in activating the immune system against **malignant** cells. The human interleukin-4 receptor (hIL-4R) is not only expressed by hematopoietic cells but also on a large

number of tissue specimens which include colon, breast and lung carcinomas. In this study we report that rhIL-4 has an antiproliferative effect on 2 out of 3 non-small cell lung carcinoma (NSCLC) cell lines in vitro as measured by human tumor cloning assays (HTCA). In comparison, rhIL-4 had no effect on the growth of small cell lung carcinoma cell lines (SCLC) in vitro. The response towards the cytokine is correlated with expression of at least 1500 high affinity receptors/cell for hIL-4 on the responsive cell lines. Xenotransplanting the human lung tumor cell lines into nude mice followed by 12 days of systemic **treatment** of the mice with rhIL-4 revealed a significant growth retardation of the IL-4R positive NSCLC cell lines when compared with the controls, whereas the growth of the IL-4R negative SCLC cell lines was unaffected also in vivo. Studies of possible mechanisms involved in the antiproliferative effect of rhIL-4 showed that rhIL-4 does not induce apoptosis or modulation of the **transcription factor** c myc in the responsive NSCLC cell lines. Additionally, the expression of the epidermal growth **factor** receptor (EGFR), which is discussed as mediating autocrine/paracrine growth stimulation of NSCLC, is unaffected by rhIL-4. However, we have observed that rhIL-4 inhibited G1-S-phase cell cycle progression. We conclude that rhIL-4 has an antiproliferative effect on the growth of some NSCLC in vitro and in vivo. The mechanisms involved remain to be further elucidated.

18/3,AB/44 (Item 44 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08743301 96094605 PMID: 7491049

Haematogenous dissemination of prostatic epithelial cells during radical prostatectomy.

Eschwege P; Dumas F; Blanchet P; Le Maire V; Benoit G; Jardin A; Lacour B ; Loric S

Service d'Urologie, Hopital de Bicetre, France.

Lancet (ENGLAND) Dec 9 1995, 346 (8989) p1528-30, ISSN 0140-6736 Journal Code: 2985213R

Comment in Lancet. 1995 Dec 9;346(8989) 1506-7; Comment in PMID 7491042; Comment in Lancet. 1996 Feb 3;347(8997):324-5; Comment in PMID 8569378; Comment in Lancet. 1996 Feb 3;347(8997):325; Comment in PMID 8569379; Comment in Lancet. 1996 Feb 3;347(8997):325-6; Comment in PMID 8569380; Comment in Lancet. 1996 Mar 30;347(9005):913; Comment in PMID 8622440

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Radical prostatectomy is one **treatment** for organ-confined prostatic adenocarcinoma. Dissemination of **malignant** prostatic cells after radical prostatectomy could be partly due to prostate manipulation during dissection. We confirmed by assay of prostate-specific membrane antigen by reverse-**transcription** nested PCR that prostate manipulation seeded prostatic epithelial cells in the general circulation in 12 of 14 consecutive patients operated on for organ-confined prostate adenocarcinoma. Our results suggest that surgeons should approach radical prostatectomy with care to avoid seeding from the prostate gland. Antiandrogen therapy might reduce the haematogenous spread of prostatic cells during radical prostatectomy.

18/3,AB/45 (Item 45 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08728213 96088838 PMID: 7489990

Hepatoma cell-specific expression of a retrovirally transferred gene is achieved by alpha-fetoprotein but not insulinlike growth **factor** II regulatory sequences.

Arbuthnot P; Bralet M P; Thomassin H; Danan J L; Brechot C; Ferry N

INSERM U370, CHU Necker-Enfants Malades, Paris, France.
Hepatology (Baltimore, Md.) (UNITED STATES) Dec 1995, 22 (6)
p1788-96, ISSN 0270-9139 Journal Code: 8302946
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

To target gene expression to **malignant** hepatic cells, we have constructed recombinant retroviral vectors containing a reporter gene encoding nuclear beta-galactosidase (nls-LacZ) under transcriptional control of regulatory sequences from the rat alpha-fetoprotein (AFP) or human insulinlike growth **factor** II (IGFII) genes. The AFP and IGFII P3 promoters activate **transcription** during fetal development and are often reactivated in hepatocellular carcinoma (HCC). Infection of several cultured cell types with the retroviral vector containing the IGFII P3 sequence resulted in expression of the reporter gene in all cell lines tested, including those that do not produce IGFII. In contrast, selective expression was achieved by vectors containing the AFP transcriptional regulatory sequence. Nuclear beta-galactosidase activity was detectable in cells from lines that produce AFP, and not in cells that do not express the AFP gene. In most infected cell lines, retroviral RNA synthesis from the 5' LTR was inhibited, and deletion of the retroviral LTR enhancer did not change expression from either the IGFII P3-nls-LacZ or the AFP-nls-LacZ cassettes. After **treatment** of cells with 12-O-tetradecanoylphorbol-13-acetate and epidermal growth **factor** (EGF), the decrease in concentrations of endogenous AFP messenger RNA (mRNA) and nls-LacZ mRNA transcribed from the transferred AFP regulatory sequence were similar. In the context of an integrated provirus, the AFP transcriptional regulatory sequence is therefore subject to similar regulatory control as that of the endogenous gene. These data show that the AFP sequence, and not the IGFII P3 promoter we used, is suitable for targeting gene expression to **malignant** hepatic cells.

18/3,AB/46 (Item 46 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08711246 96063079 PMID: 7587061

BCL-6 and the molecular pathogenesis of B-cell lymphoma.
Dalla-Favera R; Ye B H; Lo Coco F; Chang C C; Cechova K; Zhang J;
Migliazza A; Mellado W; Niu H; Chaganti S; et al
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University, New York, New York 10032, USA.

Cold Spring Harbor symposia on quantitative biology (UNITED STATES)
1994, 59 p117-23, ISSN 0091-7451 Journal Code: 1256107
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NCI

Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The results presented identify the first genetic lesion associated with DLCL, the most clinically relevant form of NHL. Although no proof yet exists of a role for these lesions in DLCL pathogenesis, the feature of the BCL-6 gene product, its specific pattern of expression in B cells, and the clustering of lesions disrupting its regulatory domain strongly suggest that deregulation of BCL-6 expression may contribute to DLCL development. A more precise definition of the role of BCL-6 in normal and neoplastic B-cell development is the goal of ongoing study of transgenic mice engineered either to express BCL-6 under heterologous promoters or lacking BCL-6 function due to targeted deletions. In addition to contributing to the understanding of DLCL pathogenesis, the identification of BCL-6 lesions may have relevant clinical implications. DLCL represent a heterogeneous group of neoplasms which are **treated** homogeneously despite the fact

that only 50% of patients experience long-term disease-free survival (Schneider et al. 1990). The fact that BCL-6 rearrangements identify biologically and clinically distinct subsets of DLCL suggests that these lesions may be useful as markers in selection of differential therapeutic strategies based on different risk groups. Furthermore, the BCL-6 rearrangements can be used to identify and monitor the **malignant** clone with sensitive PCR-based techniques. Since clinical remission has been observed in a significant fraction of DLCL cases, these markers may serve as critical tools for sensitive monitoring of minimal residual disease and early diagnosis of relapse (Gribben et al. 1993).

18/3,AB/47 (Item 47 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08669571 96017230 PMID: 7579390

Differentiation-associated changes in CD44 isoform expression during normal hematopoiesis and their alteration in chronic myeloid leukemia.

Ghaffari S; Dougherty G J; Lansdorp P M; Eaves A C; Eaves C J

Terry Fox Laboratory, British Columbia Cancer Agency, Vancouver, Canada.

Blood (UNITED STATES) Oct 15 1995, 86 (8) p2976-85, ISSN

0006-4971 Journal Code: 7603509

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

CD44 is a widely expressed, multifunctional, cell-surface glycoprotein that has been implicated in the regulation of normal hematopoiesis. In addition, expression of particular isoforms of CD44 has been associated with **malignant** transformation and/or the acquisition of metastatic potential. In this study, we used two recently developed monoclonal anti-CD44 antibodies, one reactive with an epitope shared by many CD44 isoforms and the other with an epitope unique to CD44 isoforms containing amino acids encoded by the alternatively spliced exon v10, to compare the expression of CD44 on primitive hematopoietic cells from the marrow of normal individuals and their neoplastic counterparts present in the peripheral blood of patients with chronic myeloid leukemia (CML). Multiparameter fluorescence-activated cell sorter (FACS) analysis and cell sorting studies showed that CD44 is normally expressed at high to very high levels on both long-term culture-initiating cells (LTC-IC) and granulopoietic colony-forming cells (granulocyte-macrophage colony-forming units [CFU-GM]). In contrast, primitive erythropoietic progenitors (burst-forming units-erythroid [BFU-E]) in normal marrow were more homogeneous in their expression of CD44, and very few (less than 5%) showed the very high levels of CD44 seen on 20% to 25% of LTC-IC and CFU-GM. Antibody staining showed the expression of exon v10-containing CD44 isoforms to be restricted to a small subpopulation (4% to 8%) of morphologically recognizable mature (CD34-) myeloid cells within the light-density fraction of normal marrow cells. Reverse **transcription** -polymerase chain reaction (RT-PCR) analysis showed the presence of two exon v10-containing mRNA species. In CML, a significantly greater proportion of the circulating neoplastic CFU-GM expressed very high levels of CD44, and these CFU-GM were accompanied by an increased number of light density v10+ cells, including some that coexpressed CD34. Nonmalignant hematopoietic progenitors mobilized by prior chemotherapy and growth **factor treatment** of patients with Hodgkin's disease or acute myeloid leukemia in remission showed no changes in CD44 expression relative to normal marrow progenitors. These results provide evidence of early differentiation-associated changes in CD44 expression during normal hematopoiesis in vivo that may be deregulated in the neoplastic clone of patients with CML.

18/3,AB/48 (Item 48 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

08643888 95402567 PMID: 7545621

A quantitative assay for IGF-I and IGF binding protein mRNAs: expression in **malignant** melanoma cells.

Olney R C; Anhalt H; Neely E K; Wilson D M

Department of Pediatrics, Stanford University Medical Center, CA 94305, USA.

Molecular and cellular endocrinology (IRELAND) Apr 28 1995, 110

(1-2) p213-23, ISSN 0303-7207 Journal Code: 7500844

Contract/Grant No.: DK-02175; DK; NIDDK; FD-R-000861-01; FD; FDA; HD-07626; HD; NICHD

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The quantification of messenger RNA is central in studies of gene expression. We describe a quantitative assay for specific mRNAs (QASM) that measures mRNAs for insulin-like growth **factor**-I, IGF binding proteins (IGFBPs) -2, -3, -4, and -5, and beta-actin. The assay utilizes reverse **transcription** and polymerase chain reaction, followed by an ELISA based DNA assay technique. The use of internal (competitive) quantification standards gave poorly linear results, while external standards gave linear and reproducible results. QASM results correlated with IGFBP protein concentrations in conditioned medium and with mRNA levels determined by Northern blotting. QASM was used to study IGFBP expression in human **malignant** melanoma cells. Messenger RNA for IGFBP-2, -3, and -5 were present, while IGF-I and IGFBP-4 mRNAs were not detected. IGFBP-2 and -3 expression was increased in a dose dependent manner by **treatment** with IGF-I. Protein concentrations in conditioned media paralleled mRNA levels. QASM is a sensitive, specific, and reproducible approach to determining mRNA levels.

18/3,AB/49 (Item 49 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

08642818 95401262 PMID: 7545543

c-fos is required for **malignant** progression of skin tumors.

Saez E; Rutberg S E; Mueller E; Oppenheim H; Smoluk J; Yuspa S H; Spiegelman B M

Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts 02115, USA.

Cell (UNITED STATES) Sep 8 1995, 82 (5) p721-32, ISSN 0092-8674 Journal Code: 0413066

Contract/Grant No.: HD27295; HD; NICHD

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The proto-oncogene c-fos is a major nuclear target for signal transduction pathways involved in the regulation of cell growth, differentiation, and transformation. Using the multistep skin carcinogenesis model, we have directly tested the ability of c-fos-deficient mice to develop cancer. Upon **treatment** with a tumor promoter, c-fos knockout mice carrying a v-H-ras transgene were able to develop benign tumors with similar kinetics and relative incidence as wild-type animals. However, c-fos-deficient papillomas quickly became very dry and hyperkeratinized, taking on an elongated, horny appearance. While wild-type papillomas eventually progressed into **malignant** tumors, c-fos-deficient tumors failed to undergo **malignant** conversion. Experiments in which v-H-ras-expressing keratinocytes were grafted onto nude mice suggest that c-fos-deficient cells have an intrinsic defect that hinders tumorigenesis. These results demonstrate that a member of the AP-1

family of **transcription factors** is required for the development of a **malignant** tumor.

18/3,AB/50 (Item 50 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08634046 95393413 PMID: 7664285

Expression of Pax-2 in human renal cell carcinoma and growth inhibition by antisense oligonucleotides.

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Urological Oncology Section, National Cancer Institute, Bethesda, Maryland 20892, USA.

Cancer research (UNITED STATES) Sep 15 1995, 55 (18) p4092-8,
ISSN 0008-5472 Journal Code: 2984705R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Renal cell carcinoma (RCC) is the most common malignancy in the adult kidney. Because RCC is generally thought to arise from the epithelium of the proximal tubules, the expression of Pax-2, a gene required for renal epithelium development, was examined in primary tumors and tumor-derived cell lines. Immunostaining of frozen sections from the primary tumors indicated Pax-2 expression in the **malignant** cells but not in the surrounding stroma. In a panel of human RCC-derived cell lines, 73% expressed Pax-2 protein and mRNA. **Treatment** of RCC cell lines with antisense oligodeoxynucleotides resulted in down-regulation of Pax-2 protein expression and growth inhibition after 3 days in culture. These data indicate that Pax-2 gene function is required for proliferation, as well as differentiation during embryonic development, and suggest a novel therapy for RCC.

18/3,AB/51 (Item 51 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08595809 95354138 PMID: 7627956

Thrombopoietin receptor expression in human cancer cell lines and primary tissues.

Columbyova L; Loda M; Scadden D T

Department of Medicine, New England Deaconess Hospital, Harvard Medical School, Boston, Massachusetts 02215, USA.

Cancer research (UNITED STATES) Aug 15 1995, 55 (16) p3509-12,
ISSN 0008-5472 Journal Code: 2984705R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

c-mpl is the receptor for the recently identified megakaryocyte growth and differentiation **factor** thrombopoietin. Thrombopoietin has been shown to be capable of raising platelet counts in animals and is about to enter clinical trials in humans. In anticipation of its likely use in the care of patients receiving cancer chemotherapy, we evaluated the expression of human c-mpl by reverse **transcription** PCR on 39 human cell lines and 20 primary human tissue samples derived from both normal and **malignant** sources. c-mpl transcripts were found in all megakaryocytic cell lines tested (CMK, CMK-2B, CMK-2D, SO, and DAMI), the CD34+ leukemia cell line KMT-2, and a hepatocellular carcinoma cell line (Hep3B). Among primary tissues, fetal liver cells and brain had detectable levels of c-mpl message, and among primary tumors, none were found to express c-mpl. These data support the conclusion that c-mpl has restricted expression that is primarily, but not exclusively, related to megakaryocytopoiesis. These observations suggest that thrombopoietin is unlikely to have direct effects

on other **malignant** or normal tissue should it have a clinical role in the **treatment** of chemotherapy-induced thrombocytopenia.

18/3,AB/52 (Item 52 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08565203 95323131 PMID: 7599784

Gene rearrangements and chromosomal translocations in T cell lymphoma--diagnostic applications and their limits.

Griesser H
Department of Oncologic Pathology, Ontario Cancer Institute, Toronto, Canada.

Virchows Archiv : an international journal of pathology (GERMANY)
1995, 426 (4) p323-38, ISSN 0945-6317 Journal Code: 9423843

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The diversity of the T cell receptor (TCR) repertoire is established for individual T lymphocytes by developmentally regulated gene rearrangements and shaped by predominantly intrathymic selection procedures. TCR gene probes in Southern blot experiments and TCR primers for the polymerase chain reaction (PCR) help to distinguish polyclonal from abnormal clonal T cell proliferations and to monitor clonal disease after **treatment**. Rearrangement studies can identify the lineage and developmental stage of a lymphocyte clone. Cross-lineage rearrangements, false positive or negative results are rarely misleading when morphology and immunophenotypical findings are considered. Rearrangement studies, however, have not contributed significantly to the comprehension of lymphomagenesis. Analyses of characteristic chromosomal translocations in T cell leukaemias and lymphomas may provide further insight into the mechanisms of **malignant** transformation. **Transcription factors** are often involved and sometimes abnormally transcribed, which may alter the physiological intracellular signalling in T cells. Interphase cytogenetic analysis by chromosomal fluorescence in situ hybridization (FISH) has become a new tool in the search for transformed T cells carrying specific translocations. Archival biopsy material is now accessible for PCR rearrangement studies and FISH cytogenetics. This adds another dimension to the diagnosis, disease monitoring and biological understanding of **malignant** T cell lymphomas and leukaemias.

18/3,AB/53 (Item 53 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08559793 95316848 PMID: 7540949

Mouse skin tumor progression results in differential expression of retinoic acid and retinoid X receptors.

Darwiche N; Celli G; Tennenbaum T; Glick A B; Yuspa S H; De Luca L M
Laboratory of Cellular Carcinogenesis and Tumor Promotion, National Cancer Institute, NIH, Bethesda, Maryland 20892, USA.

Cancer research (UNITED STATES) Jul 1 1995, 55 (13) p2774-82,
ISSN 0008-5472 Journal Code: 2984705R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Retinoids are powerful regulators of epidermal cell growth and differentiation and are widely used in the prevention and **treatment** of skin disorders and cancers in humans. Since many of the effects of retinoids on cell growth and differentiation are mediated by nuclear retinoid receptors (RARs and RXRs), we were interested in determining RAR and RXR gene expression during mouse skin tumor progression. The two-stage

system of mouse skin carcinogenesis was used to generate papillomas and carcinomas, and the different stages of **malignant** progression (papillomas, differentiated squamous cell carcinomas, undifferentiated squamous cell carcinomas, and spindle cell carcinomas) were characterized in each tumor by specific keratin expression prior to receptor characterization. Using in situ hybridization analysis, we show that the two major RAR isoforms (alpha 1 and gamma 1), which account for most of RARs in the skin, were expressed in both the basal and suprabasal layers in mouse epidermis. In contrast, RXR alpha transcripts were compartmentalized to the basal cell layers and concentrated in hair follicles. During skin tumor progression, RAR (alpha 1 and gamma 1) transcripts were down-modulated in **malignant** tumor cells, whereas RXR (alpha and beta) transcript expression was expanded in papillomas and carcinomas as the number of undifferentiated cells also increased. RXR gamma was not detected in the skin or at any stage during skin tumor progression. Spindle cell tumors lacked markers of the keratinocyte phenotype and lost RAR expression, yet retained expression of RXR alpha and beta. The increased abundance of transcripts for RXRs and decreased presence of RARs in skin tumor progression may favor other nuclear signal transduction pathways requiring RXR for heterodimer formation and contribute to phenotypic progression of cancer cells.

18/3,AB/54 (Item 54 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08496449 95249268 PMID: 7731708

Overexpression of the retinoic acid receptor gamma directly induces terminal differentiation of human embryonal carcinoma cells.

Moasser M M; Reuter V E; Dmitrovsky E

Department of Medicine, Memorial Hospital, New York, New York, USA.

Oncogene (ENGLAND) Apr 20 1995, 10 (8) p1537-43, ISSN

0950-9232 Journal Code: 8711562

Contract/Grant No.: 1R01-CA54494-03; CA; NCI; T32-CA-09512-10; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

All-trans retinoic acid (RA) exerts profound effects on the growth and differentiation of normal, embryonic, and **malignant** cells. The effects of RA are mediated through multiple members of the retinoic acid receptor (RAR) and retinoid X receptor (RXR) families of nuclear **transcription factors**. The RARs and RXRs exhibit specific patterns of expression during development and in adult tissues suggesting tissue or cell-type specific functions. Using NTera2/clone D1 (NT2/D1) human embryonal carcinoma cells as a model, we report that the RA induced terminal differentiation of these cells into a neuronal phenotype is characterized by an increase in expression of RAR alpha, RAR beta, RAR gamma, and a slight induction of RXR alpha. To study the role of these receptors in the differentiation process we individually overexpressed RAR alpha, beta, gamma and RXR alpha in NT2/D1 cells by cDNA transfection. Using induced cDNA expression by episomal vector amplification we show that RAR gamma over-expression causes the terminal mesenchymal differentiation of these cells while over-expression of RAR alpha, beta and RXR alpha has no observed maturation or growth inhibitory effects. Over-expression of these receptors in the derived RA resistant subclone NT2/D1-R1 showed phenotypic changes characteristic of RA response in RAR gamma transfectants. These studies indicate that of the retinoid receptors expressed in RA-treated NT2/D1 cells, it is the upregulation of RAR gamma that specifically induces the terminal differentiation of these cells.

18/3,AB/55 (Item 55 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

08445297 95189144 PMID: 7883226

1,25-Dihydroxyvitamin D3 and retinoid X receptor expression in human colorectal neoplasms.

Kane K F; Langman M J; Williams G R

Department of Medicine, University of Birmingham, Edgbaston.

Gut (ENGLAND) Feb 1995, 36 (2) p255-8, ISSN 0017-5749

Journal Code: 2985108R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Epidemiological studies suggest that 1,25-dihydroxyvitamin D3 (D3) protects against colorectal carcinogenesis. Animal and in vitro studies show an antiproliferative effect of D3 in a variety of tumours including those of large bowel origin. D3 actions are mediated by D3 receptors (VDR) alone or by VDR in conjunction with retinoid X receptors (RXRs) in all D3 responsive tissues. The expression of mRNAs encoding VDR and RXRs in normal and **malignant** human colorectum was determined. Full length VDR (4.6 kB), RXR alpha (5.5 kB), and RXR gamma (3.5 and 7 kB) mRNAs were expressed in all tissues, but RXR beta mRNA was not expressed in any. VDR expression was reduced in 12 carcinomas relative to paired normal mucosa, and RXR alpha expression was reduced in nine. There was no correlation between VDR or RXR alpha expression and the site, grade of differentiation, or Dukes's staging of the tumour. The finding of persistent VDR and RXR coexpression in all colorectal tumours provides a rational basis for exploring a role for D3 in the **treatment** of colorectal malignancy.

18/3,AB/56 (Item 56 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

08429238 95188103 PMID: 7882274

Human **malignant** melanoma. A genetic disease?

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Department of Dermatology, Ludwig-Maximilians-Universitat, Munich, Germany.

Cancer (UNITED STATES) Mar 15 1995, 75 (6) p1228-37, ISSN

0008-543X Journal Code: 0374236

Document type: Journal Article; Review; Review, Academic

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

BACKGROUND. Human hereditary **malignant** melanoma, comprising 5% of all cases of **malignant** melanoma, occurs in association with other malignancies, predominantly in families with dysplastic nevus syndrome. Additionally, higher incidences of **malignant** melanoma have been reported in individuals with genetic disorders such as ataxia telangiectasia and xeroderma pigmentosum. The results and observations as reported in the literature on the involvement of oncogenes and chromosomal aberrations in the development of **malignant** melanoma are reviewed and compared with the authors' own experimental and clinical experience. RESULTS. Numerous chromosomal regions, as on chromosomes 1 and 9, were altered. The long arm of chromosome 6 was affected in 60% of melanomas. Introduction of a normal copy of chromosome 6 resulted in loss of tumorigenicity in vitro. True melanoma genes were evident in two animal models: the Sinclair swine and the teleost fish Xiphophorus. In the Xiphophorus system, the crossing-conditioned elimination of a tumor suppressor gene led to the uncontrolled activity of a dominantly acting oncogene in certain hybrids. The causative oncogene, Xmrk, encodes a receptor tyrosine kinase closely related to human epidermal growth factor receptor (EGFR). Among the numerous studied human oncogenes, mutations in the extensively investigated ras family are the result rather

than the cause of **malignant** transformation. High expression of nuclear oncogenes simply may be a common feature of rapidly dividing cells. The receptor tyrosine kinase EGFR may be involved in late stage melanoma; the human exon with homology to Xmrk shows elevated **transcription** levels in 80% of human melanoma metastases. Deletions of the tumor suppressor gene MTS 1 may be important for melanoma formation, whereas deletions of p53 appear to be of minor relevance. **CONCLUSION.** Scientific progress in **treating** and diagnosing **malignant** melanoma will largely depend on experimental approaches to define relevant genetic changes by functional analysis rather than descriptive phenomenology and correlative observations.

18/3,AB/57 (Item 57 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08423900 95174410 PMID: 7869768

Effect of retinoic acid isomers on proliferation, differentiation and PML relocalization in the APL cell line NB4.

Zhu J; Shi X G; Chu H Y; Tong J H; Wang Z Y; Naoe T; Waxman S; Chen S J; Chen Z

Laboratory of Molecular Biology, Shanghai Institute of Hematology, Samuel Waxman Cancer Research Foundation, Laboratory, Rui-Jin Hospital, China.

Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K (ENGLAND) Feb 1995, 9 (2) p302-9, ISSN

0887-6924 Journal Code: 8704895

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Retinoic acids exert a wide physiological role in development and differentiation. Retinoic acids have also been used in the **treatment** of human cancers, particularly in acute promyelocytic leukemia (APL). A structure-function relationship of the RA isomers in terms of clinical effect has been observed since all-trans retinoic acid (ATRA) induces a high complete remission rate while 13-cis retinoic acid (13-cis RA) shows much poorer effect. In this study, we examined the effect of RA isomers, including ATRA, 13-cis RA and 9-cis RA, on the proliferation and differentiation of NB4 cells. A number of parameters such as cell growth curve, dynamics of cell cycle, expression of clusters of differentiation and reduction of nitro blue tetrazolium (NBT) as well as immunofluorescence staining of PML were used to evaluate the effects of three isomers at two concentrations (10(-8) M and 10(-7) M). It has been shown that during the first 48 h of RA **treatment**, the APL cell differentiation was coupled with the cell proliferation. Although similar effects of proliferation inhibition and differentiation induction were observed among the three isomers at 10(-7) M, significant differences appeared at a concentration of 10(-8) M, 9-cis RA showed a higher activity than that of ATRA, while ATRA showed better results than 13-cis RA. Our results provide further evidence that 9-cis RA could be a promising molecule in differentiation induction of **malignant** cells.

18/3,AB/58 (Item 58 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08420951 95166230 PMID: 7862138

The p53-mediated G1 checkpoint is retained in tumorigenic rat embryo fibroblast clones transformed by the human papillomavirus type 16 E7 gene and EJ-ras.

Peacock J W; Chung S; Bristow R G; Hill R P; Benchimol S

Ontario Cancer Institute/Princess Margaret Hospital, Toronto, Canada.

Molecular and cellular biology (UNITED STATES) Mar 1995, 15 (3)

p1446-54, ISSN 0270-7306 Journal Code: 8109087

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Rat embryo fibroblast clones transformed with the human papillomavirus type 16 E7 gene and the H-ras oncogene (ER clones) fall into two groups on the basis of endogenous p53 genotype, wild type or mutant. We have compared these clones with the aim of indentifying physiological differences that could be attributed to p53 protein function. We show that all ER clones, regardless of p53 gene status, are tumorigenic and metastatic in severe combined immunodeficiency mice. We demonstrate that only the wild-type p53 protein expressed in ER clones is functional on the basis of its site-specific double-stranded DNA-binding activity and its ability to confer a G1 delay on cells following **treatment** with ionizing radiation. These data indicate that disruption of the p53 growth-regulatory pathway is not a prerequisite for the **malignant** conversion of rat embryo fibroblasts expressing the E7 gene and mutant ras. Differences in phenotype that were correlated with loss of p53 protein function included the following: serum-independent growth of ER clones in culture, decreased tumor doubling time in vivo, and increased radioresistance. In addition, we demonstrate the p53-dependent G1 checkpoint alone does not determine radiosensitivity.

18/3,AB/59 (Item 59 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08403403 95162072 PMID: 7858536

Detection of residual leukaemia more than 10 years after allogeneic bone marrow transplantation for chronic myelogenous leukaemia.

van Rhee F; Lin F; Cross N C; Reid C D; Lakhani A K; Szydlo R M; Goldman J M

Leukaemia Research Fund Centre for Adult Leukaemia, Royal Postgraduate Medical School, London, UK.

Bone marrow transplantation (ENGLAND) Oct 1994, 14 (4) p609-12
, ISSN 0268-3369 Journal Code: 8702459

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The clinical status of a homogeneous cohort of long-term survivors of allogeneic marrow transplantation was assessed and residual leukaemia was studied by reverse **transcription** polymerase chain reaction for leukaemia specific BCR-ABL mRNA. The group comprised 34 consecutive patients with CML in chronic phase **treated** by chemoradiotherapy and transplantation of bone marrow from HLA-identical sibling donors between February 1981 and December 1983 in the joint Hammersmith-Northwick Park programme. The probability of survival at 10 years was 59 +/- 17%. Eighteen of the 19 surviving (95%) patients have Karnofsky scores of 90 or 100% indicative of a good performance status. One of the survivors had evidence of relapse 6.5 years after transplant but has since been restored to complete remission by **treatment** with interferon-alpha followed by donor leucocyte transfusions. Surprisingly, 2 of the 19 patients who have been in remission for over 10 years have molecular evidence of persisting leukaemic cells. Quantification by competitive PCR indicated that the **malignant** clone persisted at low levels. The data suggest that the majority of long-term survivors after BMT for CML are in good health and may be regarded as cured. Some long-term survivors, however, may still harbour residual leukaemic cells and continued monitoring for late relapse is warranted. Late relapse is amenable to further therapy with leukocyte transfusions from the original marrow donor.

18/3,AB/60 (Item 60 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

08391709 95130233 PMID: 7829217

Effects of cytokine-mediated modulation of nm23 expression on the invasion and metastatic behavior of B16F10 melanoma cells.

Parhar R S; Shi Y; Zou M; Farid N R; Ernst P; al-Sedairy S T

Department of Biological and Medical Research, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia.

International journal of cancer. Journal international du cancer (UNITED STATES) Jan 17 1995, 60 (2) p204-10, ISSN 0020-7136

Journal Code: 0042124

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The molecular mechanisms of tumor invasion and metastasis are yet to be fully elucidated. A potential tumor-metastasis-suppressor gene nm23 has been described in certain rodent and human tumors. In the present study, we examined the potential anti-invasive and anti-metastatic effect of nm23 gene in B16F10 cells, a **malignant** murine melanoma cell line. Transfection of nm23 gene into B16F10 melanoma cells resulted in significant suppression of the invasiveness and metastatic ability of melanoma cells and significantly enhanced the survival of tumor-bearing mice. B16F10 melanoma cells transfected with nm23 produced significantly less soluble ICAM-I and were more susceptible to LAK-cell-mediated cytotoxicity. Co-culture of B16F10 melanoma cells with IL-2 had no effect on nm23 expression, whereas **treatment** with PGE2, TNF-alpha and IFN-gamma resulted in down-regulation of nm23 expression. Concomitantly, in vivo **treatment** with TNF-alpha or IFN-gamma in experimental mice increased pulmonary metastases and lowered the overall survival period, as compared with IL-2 **treatment** alone. These results provide evidence that nm23, in addition to its anti-metastatic function, could also be involved in modulating tumor-target-structure expression, in down-regulating invasive potential and in production of soluble intracellular adhesion molecules. The down-regulation of nm23 by TNF-alpha, IFN-gamma and particularly by PGE2 warrants re-examination of current immunotherapeutic protocols and of the role played by PGE2 in tumor progression.

18/3,AB/61 (Item 61 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

08386376 95151644 PMID: 7848924

Arsenate perturbation of human keratinocyte differentiation.

Kachinskas D J; Phillips M A; Qin Q; Stokes J D; Rice R H

Department of Environmental Toxicology, University of California, Davis 95616-8588.

Cell growth & differentiation : the molecular biology journal of the American Association for Cancer Research (UNITED STATES) Nov 1994,

5 (11) p1235-41, ISSN 1044-9523 Journal Code: 9100024

Contract/Grant No.: AR27130; AR; NIAMS; ES07059; ES; NIEHS; P4204699; PHS

; +

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Treatment of cultured **malignant** human keratinocytes with sodium arsenate greatly suppressed expression of involucrin, a specific marker of keratinocyte differentiation. This action was primarily attributable to inhibition of involucrin **transcription** according to message run-on and stability measurements. Involucrin was suppressed in nontumorigenic keratinocytes as well, although the efficacy of suppression was less dramatic in cells derived from clinically normal epidermis.

Several transition metal oxyanions (vanadate, molybdate, and tungstate) also substantially suppressed involucrin expression, but okadaic acid was ineffective. Immunoblotting detected marked increases in tyrosine phosphorylation of several proteins as a consequence of arsenate **treatment** of the cultures, while mobility shift analysis revealed a dramatic loss of DNA binding by the **transcription factor** AP2. These findings support a proposed role for altered levels of protein tyrosine phosphorylation in keratinocyte differentiation. They also suggest that arsenate perturbs the differentiation program in target cells by altering this phosphorylation level and **transcription factor** activity.

18/3,AB/62 (Item 62 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08349470 95108444 PMID: 7809506

Nephroblastoma (Wilms' tumor): a model system of aberrant renal development.

Re G G; Hazen-Martin D J; Sens D A; Garvin A J
Department of Pathology, Medical University of South Carolina, Charleston 29425.

Seminars in diagnostic pathology (UNITED STATES) May 1994, 11
(2) p126-35, ISSN 0740-2570 Journal Code: 8502262

Contract/Grant No.: CA 37887; CA; NCI

Document type: Journal Article; Review; Review, Academic

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Wilms' tumor, or nephroblastoma, is a developmental malignancy of the kidney that affects approximately 1 in 10,000 children between 1 and 6 years of age. Typically, the histology of nephroblastoma reveals a disorganized renal developmental process showing blastema and epithelia randomly interspersed in varying amounts of stroma. This developmental disruption is associated with the loss of function of the tumor suppressor gene WT-1. This gene, located on chromosome 11 at band p13, codes for a zinc finger protein that may act as a transcriptional repressor. Familial cases of Wilms' tumor fit Knudson's "two hit" model, according to which a germ line mutation of one WT-1 allele predisposes to the tumor while an additional somatic mutation of the other allele causes **malignant** transformation. Originally proposed for retinoblastoma, this model defines the nature of the tumor suppressor gene as a gene that is tumorigenic when inactivated. However, not all Wilms' tumor cases fit this model because the majority of Wilms' tumors do not show a mutation of WT-1. For Wilms' tumor, the loss of tumor suppression appears to be more complex than for retinoblastoma. Some of the mechanisms recognized to date involve dominant negative WT-1 mutations, interaction of the WT-1 gene product with other mutated **transcription factors** such as p53, loss of imprinting, and mutations of other tumor suppressor genes at 11p15 or other loci. Although classic Wilms' tumor is associated with good prognosis (85% survival), its anaplastic form is often fatal. Despite the plethora of knowledge gained in recent years, Wilms' tumor remains the center of attention for further investigation because it offers opportunities for studying normal kidney development, for understanding the molecular basis for clinically important anaplastic forms, as well as for elucidating the molecular mechanisms of tumor suppressor genes. To facilitate this task, Wilms' tumor heterotransplants have been established in nude mice. This provides an indefinite source of tumor tissue and a means to test their growth properties in response to drug **treatments** or molecular genetic manipulations. Furthermore, the establishment of stable Wilms' tumor cell lines is essential to investigating further the molecular basis of tumorigenesis using recombinant DNA technology.

18/3,AB/63 (Item 63 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08348079 95106796 PMID: 7528856

Expression and regulation of CD30 ligand and CD30 in human leukemia-lymphoma cell lines.

Gruss H J; DaSilva N; Hu Z B; Uphoff C C; Goodwin R G; Drexler H G
Immunex Research and Development Corporation, Seattle, WA.

Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K (ENGLAND) Dec 1994, 8 (12) p2083-94, ISSN 0887-6924 Journal Code: 8704895

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The CD30 antigen was originally described as a specific surface marker for Hodgkin's lymphoma. Recent work established CD30 as a member of the tumor necrosis factor/nerve growth factor receptor superfamily whose ligand (CD30L) has also been cloned and expressed; CD30L is active as membrane-bound type II glycoprotein. Here, CD30L mRNA expression was studied in a panel of 102 continuous human leukemia-lymphoma cell lines and was found only in four Burkitt lymphoma, one Burkitt-type acute lymphoblastic leukemia and one non-Hodgkin's lymphoma (NHL) cell line. The product of CD30L mRNA is expressed as a membrane protein on the surface of these malignant B-cell lines. Treatment of these cell lines with soluble CD27L, phorbol ester or staphylococcus aureus Cowan antigen resulted in the enhancement of cell surface CD30L protein expression. CD30L mRNA was not detected in normal unstimulated peripheral blood (PB) monocytes, monocyte-derived macrophages, or T-cells, but was detected in primary granulocytes; exposure to activating reagents induced and upregulated CD30L transcription in these different PB populations. While CD40 and CD30L surface protein expression on PB monocytes could be enhanced or induced by treatment with gamma-interferon, these cells remained negative for CD30, both at the mRNA and at the protein level. Similarly, PB monocyte-derived macrophages and granulocytes remained negative for CD30 mRNA and protein expression, regardless of stimulation. Only activated T-cells expressed CD30 mRNA and surface protein. CD30L-transfected cells and cells constitutively expressing CD30L delivered a similar stimulus for proliferation of the CD30+ Hodgkin's disease (HD)-derived cell line HDLM-2, but inhibited proliferation of the CD30+ large cell anaplastic lymphoma cell line KARPAS-299. These data provide strong evidence for the involvement in growth regulation of recombinant and natural CD30L through its interaction with the CD30 receptor. Collectively, these data suggest that the CD30L-CD30 interaction has potent biological activity and might play a critical role in the immune response and pathogenesis of HD and some NHL, in particular Burkitt lymphomas.

18/3,AB/64 (Item 64 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08343343 95100704 PMID: 7802423

[Etiopathogenesis of pituitary tumors]

Etiopathogenie des tumeurs hypophysaires.

Morange-Ramos I; Pellegrini I; Caccavelli L; Brue T; Enjalbert A; Jaquet P

Laboratoire ICNE-UMR 9941, Faculte de Medecine du Nord, Marseille.

Annales d'endocrinologie (FRANCE) 1994, 55 (1) p25-32, ISSN 0003-4266 Journal Code: 0116744

Document type: Journal Article; Review; Review, Academic ; English Abstract

Languages: FRENCH

Main Citation Owner: NLM

Record type: Completed

In this present work, the authors discuss some recent advances in the pathogenesis of pituitary tumours. The model of transgenic mice suggest that chronic hormonal stimulation and some growth **factors** could sustain pituitary tumour development. However, these data are not suitable for human pituitary adenomas. The evidence that most pituitary adenomas are monoclonal in origin has prompted a search for somatic mutations. The mutated Gs alpha are found in only 30-40% of somatotroph adenomas and the ras mutations seem to be associated with the **malignant** transformation. In some prolactinomas resistant to the bromocriptine **treatment**, quantitative and qualitative alterations of the dopamine receptor D2, have been described. Mutations of protein kinase C have been identified in some invasive pituitary tumours. Molecular abnormalities have been reported in some cases (allele loss at the 11q13 locus, retinoblastoma gene mutation, aberrant expression of hst gene, Pit-1 overexpression) but none by itself can explain the tumour formation. The pituitary tumorigenesis is certainly a multistep process with the intervention of multiple promoting **factors**.

18/3,AB/65 (Item 65 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08320150 95077478 PMID: 7986144

Detection of submicroscopic lymph node metastases with polymerase chain reaction in patients with **malignant** melanoma.

Wang X; Heller R; VanVoorhis N; Cruse C W; Glass F; Fenske N; Berman C; Leo-Messina J; Rappaport D; Wells K; et al

Cutaneous Oncology Program, Moffitt Cancer Center, University of South Florida, Tampa.

Annals of surgery (UNITED STATES) Dec 1994, 220 (6) p768-74,
ISSN 0003-4932 Journal Code: 0372354

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

BACKGROUND. The presence or absence of lymph node metastases in patients with **malignant** melanoma is the most powerful prognostic **factor** for predicting survival. If regional nodal metastases are found, the 5-year survival for the patient decreases approximately 50%. If the presence or absence of regional nodal metastases will determine which patients receive formal dissections or which patients enter adjuvant trials, then a technique is needed to accurately screen lymph node samples for occult disease. Routine histopathologic examination routinely underestimates the number of patients with metastases. This study was initiated to develop a highly sensitive clinically applicable method to detect micrometastases by examining lymph nodes for the presence of tyrosinase messenger RNA (mRNA). The hypothesis was that if mRNA for tyrosinase is found in the lymph node preparation, that finding is good evidence that metastatic melanoma cells are present. **METHODS.** The assay is accomplished using the combination of reverse **transcription** and double-round polymerase chain reaction (RT-PCR). The amplified samples are examined on a 2% agarose gel and tyrosinase cDNA is seen as a 207 base pair fragment. Lymph node preparations from 29 patients who were clinically stage I and II and undergoing elective node dissections were analyzed both by standard pathologic staining and RT-PCR. **RESULTS.** Eleven of 29 lymph node (38%) samples from 29 patients with intermediate thickness melanoma were pathologically positive. Nineteen of the 29 lymph node preparations (66%) were RT-PCR-positive, and these included all of the pathologically positive samples, so that the false-negative rate was 0. In a spiking experiment, one SK-Mel-28 melanoma cell in a background of one million normal lymphocytes could be detected, thus indicating the sensitivity of this method. In addition, analysis by restriction enzyme mapping showed that the amplified 207-bp PCR product produced is part of the tyrosinase gene sequence. **CONCLUSION.** The RT-PCR method is an extremely sensitive,

reproducible, and efficient technique for the identification of micrometastases in patients with melanoma and could be widely applicable. If clinical correlation is obtained, staging of the melanoma patient becomes more accurate, and **treatment** becomes more standardized and rational, because all those patients who have evidence of nodal disease can be identified so that they may benefit from more extensive surgery (formal node dissections) or adjuvant therapies. Based on these results, RT-PCR could be a powerful tool to detect micrometastatic melanoma.

18/3,AB/66 (Item 66 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08289673 95047455 PMID: 7958974

Triplex formation at the rat neu oncogene promoter.

Gee J E; Yen R L; Hung M C; Hogan M E

Center for Biotechnology, Baylor College of Medicine, The Woodlands, TX 77381.

Gene (NETHERLANDS) Nov 4 1994, 149 (1) p109-14, ISSN

0378-1119 Journal Code: 7706761

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Current cancer chemotherapy **treatments** generally act by affecting rapidly growing **malignant** cells. Unfortunately, they are relatively nonspecific and thus have a tendency to affect other rapidly growing normal cells in a deleterious manner. Triplex-forming oligodeoxyribonucleotides (TFOs) promise to be a new class of sequence-specific DNA-binding drugs which will target malignancies at the transcriptional level. The formation of an intermolecular triplex (triple helix) has been shown to block the binding of **transcription factors** and repress **transcription** in genes such as c-myc and that encoding the epidermal growth **factor** receptor. The rat neu oncogene promoter contain promoter-enhancer elements which are purine/pyrimidine rich. These enhancer elements are amenable to targeting by TFOs. the human counterpart of rat neu, HER2, is often found to be amplified or overexpressed in a variety of malignancies, such as those of the breast, lungs, ovary, colon and stomach. TFOs may proved to be the basis of effective chemotherapy drugs for these cancers. TFO binding at the "GTG" element (5'GGTGGGGGGG) and at the 'GA' element (5'GGAGGAGGAGGG) has been characterized by gel mobility shift analysis and DNase 1 footprinting. Binding has been shown to occur at a Kd as low as 10(-8) M and has been shown to be sequence specific.

18/3,AB/67 (Item 67 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08277295 95036240 PMID: 7524739

Growth of erythroid colonies in chronic myelogenous leukemia is independent of erythropoietin only in the presence of steel **factor**.

Issaad C; Vainchenker W

INSERM U 362, Institut Gustave Roussy, Villejuif, France.

Blood (UNITED STATES) Nov 15 1994, 84 (10) p3447-56, ISSN

0006-4971 Journal Code: 7603509

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The mechanisms of the chronic myeloid leukemia (CML) clones proliferative advantage over normal clones are currently unknown. They may involve an insensitivity to a negative regulation of a growth **factor**-independent proliferation. Clonogenic progenitors from CML patient blood or marrow in chronic phase were grown either in the presence or absence of recombinant

growth factors. No erythroid colonies were observed in the absence of any cytokine. In contrast, erythroid colonies composed of fully mature hemoglobinized erythroblasts (day 12 burst-forming units-erythroid) were obtained in the presence of Steel factor (SF) alone. Addition of erythropoietin (Epo) to SF either had no effect on the cloning efficiency or increased up to 50% the number of erythroid colonies. No erythroid growth was observed when cultures were stimulated by interleukin-3 or granulocyte-macrophage colony-stimulating factor alone. Similar erythroid growth in the presence of SF but without Epo was obtained in "serum-free" cultures when purified blood CML CD34+ cells were grown. This growth of erythroid colonies in the absence of Epo was not accounted for by an autocrine stimulation loop by Epo, because neutralizing antibodies against Epo did not inhibit it. This abnormal response to growth factor was specifically observed in the CML clone, as shown by the presence of the BCR-ABL transcript in all of these erythroid colonies. The direct implication of BCR-ABL was further documented (1) by studies of alpha-interferon-treated patients with a chimerism in which the abnormal growth correlates with the presence of the malignant clone and (2) by the use of antisense oligonucleotide against BCR-ABL transcript, which abrogated this abnormal growth. Finally, erythroid growth in the SF presence was greatly diminished by herbimycin A, whereas, at the same concentration, this tyrosine kinase inhibitor had no marked effect on erythroid colony formation in the presence of SF plus Epo on CML or normal marrow cells. This result suggests that the BCR-ABL kinase activity leads directly to this Epo-independent terminal differentiation requiring, however, the presence of SF.

18/3,AB/68 (Item 68 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08204785 94340171 PMID: 7520332

Detection of nuclear retinoic acid receptor mRNA in histological tissue sections using nonradioactive in situ hybridization histochemistry.

Xu X C; Clifford J L; Hong W K; Lotan R

Department of Tumor Biology, University of Texas M.D. Anderson Cancer Center, Houston.

Diagnostic molecular pathology : the American journal of surgical pathology, part B (UNITED STATES) Jun 1994, 3 (2) p122-31, ISSN 1052-9551 Journal Code: 9204924

Contract/Grant No.: PO1 CA52051; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Nuclear retinoic acid receptors (RARs) function as ligand-activated trans-acting transcription factors and mediate the effects of retinoids on gene expression, cell growth, and differentiation. Determination of the receptors' expression in premalignant and malignant lesions may provide prognostic value and direct the selection of receptor-specific retinoids in cancer prevention or treatment. We describe a sensitive and practical in situ hybridization method for the analysis of RARs in tissue sections of fixed and embedded surgical specimens. Digoxigenin-labeled antisense and sense RNA probes were prepared for nuclear RAR-alpha, RAR-beta, and RAR-gamma. The specificity of the probes for their respective receptor mRNAs was demonstrated by Northern blot hybridization to total RNA extracted from murine and human cells. Optimal conditions for in situ localization of the RAR mRNA were established using cultured tumor cells, and these conditions were then used for the detection of RAR mRNA in formalin-fixed, paraffin-embedded sections of surgical specimens from human tumors. The hybridization stain was detected in the cytoplasm (where it was expected to be localized) and not seen in the cell nucleus. This method provides a rapid detection procedure with good resolution that allows one to clearly

distinguish strongly and weakly stained cells. A comparison of receptor expression in head and neck squamous carcinoma specimens and in adjacent normal tissues revealed a significant decrease in the level of RAR-beta mRNA in the tumor cells.

18/3,AB/69 (Item 69 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08182285 94318462 PMID: 8043432

Regulation of **transcription factors** NF kappa B and AP-1 following tumour necrosis **factor**-alpha **treatment** of cells from chronic B cell leukaemia patients.

Jabbar S A; Hoffbrand A V; Gitendra Wickremasinghe R
Department of Haematology, Royal Free Hospital School of Medicine, London.

British journal of haematology (ENGLAND) Mar 1994, 86 (3)
p496-504, ISSN 0007-1048 Journal Code: 0372544

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Malignant B lymphocytes from patients with B-chronic lymphocytic leukaemia (B-CLL) or hairy cell leukemia (HCL) are refractory in vitro to mitogenic stimulation by several agents which trigger proliferation of normal B cells. Tumour necrosis **factor** (TNF) is a growth **factor** for these **malignant** cells, although the proliferative response is usually small. TNF regulates some of its cellular responses via induction of the **transcription factors** NF kappa B and AP-1 (jun/fos). The induction of NF kappa B by TNF is mediated via a novel signalling pathway involving the generation of reactive oxidative intermediates. Induction of jun and fos proteins (polypeptide components of AP-1) are mediated via pathways involving protein kinase C and the protein kinase encoded by the raf proto-oncogene. Here we have used an electrophoretic mobility shift assay to show that TNF induced NF kappa B in **malignant** cells isolated from 3/3 HCL and 15/15 B-CLL patients. By contrast, phorbol myristate acetate (PMA), a direct activator of protein kinase C, failed to activate this **transcription factor** in 1/1 HCL and 5/5 B-CLL isolates. The induction of jun and fos proteins (as detected by Western blot analysis) showed greater heterogeneity. Nuclear jun was induced by TNF in 5/12 chronic B cell leukaemia isolates. PMA induced this protein in 4/5 samples. Nuclear fos was induced by TNF in only 2/12 isolates and by PMA in 2/5. The data suggest that the pathways for the activation of jun and fos by TNF are defective in some B-CLL and HCL cells and that these defects may be heterogeneous. The induction of AP-1 is crucial in securing the mitogenic response to TNF. It is therefore plausible that these lesions may contribute to the refractory nature of B-CLL and HCL cells to proliferative stimuli in vitro.

18/3,AB/70 (Item 70 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08135960 94268527 PMID: 8208268

Rearrangement of the bcl-6 gene as a prognostic marker in diffuse large-cell lymphoma.

Offit K; Lo Coco F; Louie D C; Parsa N Z; Leung D; Portlock C; Ye B H; Lista F; Filippa D A; Rosenbaum A; et al

Cell Biology and Genetics Program, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

New England journal of medicine (UNITED STATES) Jul 14 1994, 331

(2) p74-80, ISSN 0028-4793 Journal Code: 0255562

Contract/Grant No.: CA-08748; CA; NCI; CA-34775; CA; NCI; CA-44029; CA; NCI

Comment in N Engl J Med. 1994 Jul 14;331(2) 116-8; Comment in PMID 8208254

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

BACKGROUND. About 40 percent of non-Hodgkin's lymphomas are diffuse lymphomas with a large-cell component (DLCL). Current therapy can induce a long-term remission in half the patients with DLCL, but more intensive **treatment** has the potential to improve outcome, particularly in patients at high risk for **treatment** failure. Clinical and cytogenetic markers can identify subgroups at high or low risk. Rearrangement of a novel candidate proto-oncogene, bcl-6, is a possible prognostic indicator in DLCL. METHODS. We performed Southern blot hybridization to detect bcl-6 and bcl-2 gene rearrangement in samples of lymphoma from 102 patients with B-cell DLCL. The results were correlated with the patients' histologic features, age, disease stage, tumor sites and bulk of disease, serum lactate dehydrogenase level, and **treatment** outcome. RESULTS. Rearranged bcl-6 was found in 23 cases, and rearranged bcl-2 in 21 cases. Nineteen of the patients with rearranged bcl-6 had extranodal DLCL, two had primary splenic lymphomas, and only one had bone marrow involvement. Thirty-six months after diagnosis, the proportion with freedom from progression of disease was projected to be 82 percent (95 percent confidence interval, 66 to 98 percent) among the patients with rearranged bcl-6, as compared with 56 percent (95 percent confidence interval, 43 to 70 percent) for the patients with germ-line bcl-6 and bcl-2 and 31 percent (95 percent confidence interval, 8 to 53 percent) for the patients with rearranged bcl-2. The status of the bcl-6 gene was an independent prognostic marker of survival and freedom from disease progression in a multivariate model and added predictive value to established prognostic signs. CONCLUSIONS. Rearrangement of the bcl-6 gene correlated with a favorable clinical outcome in DLCL and may thus serve as a prognostic marker in patients with this form of **malignant** lymphoma.

18/3,AB/71 (Item 71 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08131976 94276610 PMID: 7516458

Deranged activity of the CD44 gene and other loci as biomarkers for progression to metastatic malignancy.

Tarin D; Matsumura Y

Nuffield Department of Pathology, Oxford University, John Radcliffe Hospital, Headington, England.

Journal of cellular biochemistry. Supplement (UNITED STATES) 1993
17G p173-85, ISSN 0733-1959 Journal Code: 8207539

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

About one in three people in modern industrialised countries die of the consequences of **malignant** tumours or are found to carry an unsuspected one at the time of autopsy. Early resection of such lesions and appropriate adjuvant therapy is very effective in curing the disease. There is therefore a strong clinical incentive to find effective methods of early diagnosis, assessment of prognosis and **treatment** of neoplastic lesions and research on this topic is directed at a numerically significant medical problem. Recently it has been found that many human tumours show severe abnormalities in the expression of the CD44 gene which increase with progression to metastatic malignancy. By alternative splicing mechanisms this gene codes for a family of heavily glycosylated cell surface proteins involved in many important cellular activities. In neoplasia there is gross overexpression of various products of the gene associated with disorderly splicing, which can be detected in clinical samples with the sensitive

technique of reverse **transcription** -polymerase chain reaction (RT-PCR). These disturbances begin early in the neoplastic process and can be detected in very small biopsy samples. It has also been shown that it is possible to achieve non-invasive diagnosis of malignancy by analysis of CD44 expression in exfoliated cells in body fluids and waste products. The potential significance of these observations for early diagnosis of symptomatic cancer and for screening of the population for asymptomatic lesions are readily seen and await further investigation. Separate work in our laboratory has succeeded in DNA-mediated transfer of metastatic capability through two rounds of transfection into non-metastatic tumour cells and a metastasis-associated human DNA fragment has been recovered from the transfectants and sequenced. Using primers designed to anneal to a coding region identified by computer analysis within the novel sequence, it has been shown with RT-PCR that it is heavily expressed in metastatic cancer tissues, but not in corresponding normal ones. This could be of value in assessing the prognosis of patients using small biopsy samples from their primary tumours and the potential of this sequence for such purposes and for possible therapeutic intervention is currently being explored. Recent work in several laboratories has shown that elevated expression of certain other specific growth **factor** genes, including c-met and EGFR, correlates with metastatic capability. Combined evaluation of such markers in further studies will in time give useful information on the prognosis of individual patients to guide therapeutic decisions and the implications of these recent advances for clinical practice and future research are discussed below.

18/3,AB/72 (Item 72 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08123625 94252245 PMID: 8194469

Effects of transforming growth **factor** -beta on parathyroid hormone-related protein production and ribonucleic acid expression by a squamous carcinoma cell line in vitro.

Merryman J I; DeWille J W; Werkmeister J R; Capen C C; Rosol T J

Department of Veterinary Pathobiology, Ohio State University, Columbus 43210.

Endocrinology (UNITED STATES) Jun 1994, 134 (6) p2424-30,
ISSN 0013-7227 Journal Code: 0375040

Contract/Grant No.: AR40220; AR; NIAMS; CA-08688; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The effects of human recombinant transforming growth **factor** (TGF)-beta 1 were determined on PTH-related protein (PTHrP) production and messenger RNA (mRNA) expression by a canine squamous carcinoma cell line (SCC 2/88) in vitro. TGF-beta increased PTHrP production in a dose- and time-dependent manner ($P < 0.05$) as measured by RIA, and the effects of TGF-beta **treatment** persisted up to 72 h after removal. TGF-beta increased PTHrP production by SCC 2/88 cells until cellular confluence, at which time there was no longer a significant increase compared to control. Actinomycin D inhibited the TGF-beta-mediated increase in PTHrP production, suggesting that TGF-beta acted in part by increasing gene **transcription**. SCC 2/88 cells also produced active TGF-beta as measured by a [3H]thymidine incorporation assay in mink lung epithelial cells. Exposure of SCC 2/88 cells to a neutralizing anti-TGF-beta monoclonal antibody decreased (up to 50%, $P < 0.01$) basal PTHrP production. TGF-beta increased PTHrP mRNA expression in a dose- and time-dependent manner as evaluated by northern blot analysis. Postconfluent SCC 2/88 cells expressed little mRNA for PTHrP, and there was only a minimal increase in PTHrP mRNA expression in postconfluent TGF-beta-**treated** cells. These results indicate that TGF-beta increased PTHrP production and mRNA expression in **malignant** keratinocytes and suggest that TGF-beta may

be an important **factor** in the pathogenesis of humoral hypercalcemia of malignancy.

18/3,AB/73 (Item 73 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08080529 94215207 PMID: 8162612

In vivo regulation of transforming growth **factor** beta 1 **transcription** by immunotherapy: interleukin-2 impairs interferon-alpha-stimulated increase in steady-state mRNA levels of transforming growth **factor** beta 1.

Jahn B; Brieger J; Fenchel K; Mitrou P S; Bergmann L
Department of Internal Medicine, J.W. Goethe University Frankfurt/M, Germany.

Cancer immunology, immunotherapy : CII (GERMANY) May 1994, 38

(5) p304-10, ISSN 0340-7004 Journal Code: 8605732

Document type: Clinical Trial; Clinical Trial, Phase II; Journal Article
Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Recombinant interleukin-2 (rIL-2) in combination with recombinant interferon alpha (rIFN alpha) has been shown to mediate significant antitumoral effects in some patients with advanced renal cell cancer or **malignant** melanoma. The therapeutic effects may be partially modulated by secondarily induced cytokines, especially with regard to in vivo lymphocyte activation. To investigate possible negative effects on lymphocyte activation during immunotherapy, we designed a study on **transcription** of transforming growth **factor** beta 1 (TGF beta 1), a known inhibitor of lymphocyte function, in patients undergoing **treatment** with daily alternating administration of rIFN alpha and rIL-2. Here we present data on gene expression of TGF beta 1. Kinetic mRNA studies revealed an increase of TGF beta 1 mRNA in peripheral mononuclear cells 12 h after subcutaneous injection of rIFN alpha. The following intravenous rIL-2 administration significantly decreased the amounts of TGF beta 1-specific mRNA. In contrast to the effect of the first dose, subsequent application of rIFN alpha did not enhance TGF beta gene expression during rIFN alpha/IL-2 therapy. The diminished TGF beta 1 gene expression returned to pretreatment levels 1-7 days after the last rIL-2 administration. When concomitant with a decrease in TGF beta 1 transcripts. Our results indicate a complex regulatory effect on secondarily induced cytokines such as TGF beta 1 by immunotherapeutic approaches. The rIL-2-mediated down-regulation of increased TGF beta 1 steady-state mRNA levels following rIFN alpha may represent a positive immune regulatory effect on cytotoxic cells. Furthermore this effect may modulate proliferation of neoplastic tissues.

18/3,AB/74 (Item 74 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08055554 94185059 PMID: 8137325

Suppression of squamous cell carcinoma growth and differentiation by retinoids.

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Cancer research (UNITED STATES) Apr 1 1994, 54 (7 Suppl)

p1987s-1990s, ISSN 0008-5472 Journal Code: 2984705R

Contract/Grant No.: PO1-52051; PHS

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The epithelium of the oral cavity is mostly nonkeratinizing. However, it undergoes an abnormal squamous differentiation with keratinization during vitamin A deficiency or oral carcinogenesis. Vitamin A analogues (retinoids) were found to be effective in preventing oral premalignant lesions and second primary cancers in the upper aerodigestive tract. Further development of retinoids for prevention and therapy of squamous cell carcinoma (SCC) requires a better understanding of their mechanism action on the growth and differentiation of SCC cells. We used cultured head and neck SCC (HNSCC) cell lines as a model system. **Treatment** of HNSCC cells with beta-all-trans-retinoic acid resulted in inhibition of growth (proliferation and colony formation) and suppression of squamous differentiation to varying degrees in the different cell lines. Because some of the **malignant** HNSCC cells recapitulate the main characteristics of keratinocyte squamous differentiation and responsiveness to retinoids, they can serve as a model for investigating the mechanism underlying the effects of retinoids on cell growth and differentiation. It is thought that nuclear retinoic acid receptors (RARs) and retinoid X receptors (RXRs) mediate the above effects of retinoids by acting as DNA-binding **transcription-modulating factors**. We found that HNSCC cell lines express several nuclear RAR and that their level could be modulated by retinoids in some cell lines. An inverse relationship was found between RAR-beta expression and squamous differentiation. An analysis of RAR mRNA expression in head and neck cancer specimens revealed a decrease in RAR-beta in premalignant and **malignant** tissues relative to normal mucosa. The expression of this receptor increased in vivo after **treatment** with 13-cis-retinoic acid. These results implicate the loss of RAR-beta expression in the development of head and neck cancer and suggest that RAR-beta could serve as an intermediate marker in prevention trials.

18/3,AB/75 (Item 75 from file: 155)
 DIALOG(R) File 155:MEDLINE(R)

08024476 94162160 PMID: 7509621

BCNU-resistant human glioma cells with over-representation of chromosomes 7 and 22 demonstrate increased copy number and expression of platelet-derived growth **factor** genes.

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Neuro-Oncology Research, Barrow Neurological Institute of St. Joseph's Hospital, Phoenix, AZ 85013.

Genes, chromosomes & cancer (UNITED STATES) Nov 1993, 8 (3)

p137-48, ISSN 1045-2257 Journal Code: 9007329

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Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

We used standard karyotypic analyses of first-division cells to identify a subpopulation of cells in primary **malignant** gliomas with over-representation of chromosomes 7 and 22. These cells are a minor subpopulation in the primary tumor but become the dominant population after **treatment** in vitro of the cells with the chemotherapeutic agent 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU). The selection for a cell with this specific karyotypic abnormality suggests that these chromosomes contain genes important to the growth of BCNU-resistant cells. Southern blot hybridization analyses demonstrate an increased copy number of the genes encoding platelet-derived growth **factor** (PDGF) A-chain and B-chain, which have been mapped to chromosomes 7 and 22, respectively. Reverse **transcription** followed by polymerase chain reaction (RT-PCR) analysis demonstrates increased expression of these genes. In addition, these cells secrete a mitogenic **factor** that stimulates 3H-thymidine uptake in NIH 3T3 cells. This **factor** is sensitive to anti-PDGF antibodies and beta-mercaptoethanol, but not to anti-EGF antibodies. These

data suggest that autocrine and/or paracrine mechanisms occur in human **malignant** gliomas, and that over-expression of PDGF may play a role in the growth of BCNU-resistant cells in these tumors.

18/3,AB/76 (Item 76 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08018370 94162147 PMID: 8117615

Coordinate regulation of collagen II(alpha 1) and H19 expression in immortalized hamster cells.

Owen R D; Hosoi J; Montgomery J C; Wiseman R; Barrett J C
Laboratory of Molecular Carcinogenesis, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, North Carolina 27709.

Cell growth & differentiation : the molecular biology journal of the American Association for Cancer Research (UNITED STATES) Dec 1993,

4 (12) p1013-21, ISSN 1044-9523 Journal Code: 9100024

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Loss of tumor suppressor gene function is essential in the multistep progression of cells to neoplasia. Immortalized cells were established by carcinogen **treatment** of Syrian hamster embryo cells. At early passages, these nontumorigenic cells retained the ability to suppress tumorigenicity in cell hybrids with **malignant** cells. Upon passage and subcloning of these suppressor-positive (supB+) cells, variant clones that had lost tumor suppressor activity were isolated. These suppressor-negative (supB-) clones remained nontumorigenic. The mRNAs encoding collagen II(alpha 1a), a chondrocyte differentiation marker, and H19, a developmentally controlled gene, were more abundant in supB+ cells than in supB- cells. Nuclear run-on analysis indicated that the **transcription** of these genes is differentially regulated. Transient transfection experiments revealed that a cis-acting element in the rat collagen II 5' flanking sequences directs differentially regulated **transcription**. Gel retention analysis demonstrated the presence of a nuclear DNA-binding **factor** (s) that specifically recognizes a DNA sequence common to both the rat collagen II sequences and the mouse H19 enhancer. In one set of clones, transcriptional regulation could account for differential collagen II and H19 expression in supB+ and supB- cells. In another set of clones, posttranscriptional controls are responsible for the decreased expression of these genes in supB- cells. The emergence of two independent mechanisms that cause differential expression of collagen II and H19 related to tumor suppressor loss suggests that coordinate regulation of these genes, or others regulated by common mechanisms, may be important in tumor suppression.

18/3,AB/77 (Item 77 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07981217 94129776 PMID: 1285293

Platelet-derived growth **factor** gene expression in the kidney of **malignant** hypertension.

Okamura M; Konishi Y; Nishimura M; Negoro N; Umetani N; Inoue T; Kanayama Y; Takeda T

First Department of Internal Medicine, Osaka City University Medical School, Japan.

Blood pressure. Supplement (NORWAY) 1992, 3 p17-21, ISSN 0803-8023 Journal Code: 9300787

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

To examine the pathogenetic role of platelet-derived growth factor (PDGF) in hypertensive kidney damage, we studied the gene expression of PDGF A-chain and B-chain in an animal model of **malignant** hypertension. Experimental **malignant** hypertension induced by unilateral nephrectomy combined with deoxycorticosterone and salt loading in the spontaneously hypertensive rat resulted in severely elevated blood pressure and renal histological damage, characterized by necrotizing vasculitis. Using reverse **transcription**-polymerase chain reaction analysis followed by Southern blot analysis, we observed that PDGF B-chain gene expression was increased in the kidney of experimental **malignant** hypertension and was correlated with the severity of glomerular damage, while PDGF A-chain gene expression was unaffected. Antihypertensive **treatment** with manidipine reduced glomerular damage and a decreased gene expression of PDGF B-chain. These results suggest that PDGF B-chain may have a role in mediating hypertensive kidney damage.

18/3,AB/78 (Item 78 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07975798 94088580 PMID: 8264646

MGSA/GRO **transcription** is differentially regulated in normal retinal pigment epithelial and melanoma cells.

Shattuck R L; Wood L D; Jaffe G J; Richmond A

Veterans Affairs Medical Center, Nashville, Tennessee 37212-2637.

Molecular and cellular biology (UNITED STATES) Jan 1994, 14 (1)

p791-802, ISSN 0270-7306 Journal Code: 8109087

Contract/Grant No.: AR07491; AR; NIAMS; CA56704; CA; NCI; R29-EYO 9106-1; EY; NEI

Erratum in Mol Cell Biol 1995 Feb;15(2) 1136

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

We have characterized constitutive and cytokine-regulated MGSA/GRO alpha, -beta, and -gamma gene expression in normal retinal pigment epithelial (RPE) cells and a **malignant** melanoma cell line (Hs294T) to discern the mechanism for MGSA/GRO constitutive expression in melanoma. In RPE cells, constitutive MGSA/GRO alpha, -beta, and -gamma mRNAs are not detected by Northern (RNA) blot analysis although nuclear runoff experiments show that all three genes are transcribed. In Hs294T cells, constitutive MGSA/GRO alpha expression is detectable by Northern blot analysis, and the level of basal MGSA/GRO alpha **transcription** is 8- to 30-fold higher than in RPE cells. In contrast, in Hs294T cells, basal MGSA/GRO beta and -gamma **transcription** is only twofold higher than in RPE cells and no beta or gamma mRNA is detected by Northern blot. These data suggest that the constitutive MGSA/GRO alpha mRNA in Hs294T cells is due to increased basal MGSA/GRO alpha gene **transcription**. The cytokines interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF alpha) significantly increase the mRNA levels for all three MGSA/GRO isoforms in Hs294T and RPE cells, and both transcriptional and posttranscriptional mechanisms are operational. Nuclear runoff assays indicate that in RPE cells, a 1-h IL-1 **treatment** induces a 10- to 20-fold increase in **transcription** of MGSA/GRO alpha, -beta and -gamma but only a 2-fold increase in Hs294T cells. Similarly, chloramphenicol acetyltransferase (CAT) reporter gene analysis using the MGSA/GRO alpha, -beta, and -gamma promoter regions demonstrates that IL-1 **treatment** induces an 8- to 14-fold increase in CAT activity in RPE cells but only a 2-fold increase in Hs294T cells. The effect of deletion or mutation of the MGSA/GRO alpha NF-kappa B element, combined with data from gel mobility shift analyses, indicates that the NF-kappa B p50/p65 heterodimer in RPE cells plays an important role in IL-1- and TNF alpha-enhanced gene **transcription**. In Hs294T cells, gel shift analyses indicate that IL-1

and TNF alpha induce NF-kappa B complex formation; however, transactivation does not occur, suggesting that subtle differences in the NF-kappa B complexes may result in the inability of the cytokines IL-1 and TNF alpha to activate **transcription** of the MGSA/GRO genes. IL-1 and TNF alpha posttranscriptionally regulate MGSA/GRO mRNA levels in both cell types. In Hs294T cells, IL-1 increases the half-life of MGSA/GRO alpha from 15 min to 6 h (a 24-fold increase in half-life). (ABSTRACT TRUNCATED AT 400 WORDS)

18/3,AB/79 (Item 79 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07910262 94047132 PMID: 8230261

Phase I study of bryostatin 1: assessment of interleukin 6 and tumor necrosis **factor** alpha induction in vivo. The Cancer Research Campaign Phase I Committee.

Philip P A; Rea D; Thavas P; Carmichael J; Stuart N S; Rockett H; Talbot D C; Ganesan T; Pettit G R; Balkwill F; et al

Imperial Cancer Research Fund Clinical Oncology Unit, Churchill Hospital, Oxford, England.

Journal of the National Cancer Institute (UNITED STATES) Nov 17 1993, 85 (22) p1812-8, ISSN 0027-8874 Journal Code: 7503089

Comment in J Natl Cancer Inst. 1993 Nov 17;85(22) 1790-2; Comment in PMID 8230254

Document type: Clinical Trial; Clinical Trial, Phase I; Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

BACKGROUND: Many oncogenes have been shown to code for growth **factor** receptors that are involved in regulation of cell growth and proliferation and can activate **transcription** via protein kinase C. Bryostatin 1, a partial agonist of protein kinase C, has demonstrated potent antitumor activity in vitro and in vivo in human tumor xenografts. PURPOSE: The aim of this phase I study was to determine the optimal dosage and toxicity profile of bryostatin 1 and its influence on cytokine release in vivo. METHODS: Three successive cohorts consisting of 35 patients with various **malignant** tumors were **treated** with bryostatin 1 by intravenous infusion over 1 hour as follows: cohort A--35 micrograms/m2 (three patients) or 50 micrograms/m2 (eight patients) once every 2 weeks; cohort B--25 micrograms/m2 once a week (eight patients); and cohort C--25 micrograms/m2 once a week for 3 weeks, with no **treatment** during the 4th week (16 patients). Plasma levels of tumor necrosis **factor** alpha (TNF-alpha) and interleukin 6 (IL-6) were measured by immunoradiometric assay and by radioimmunoassay, respectively. RESULTS: The dose-limiting toxicity was grade 3 or 4 myalgia in four of 11 patients in cohort A, in two of eight in cohort B, and in none of 16 in cohort C. Occurrence of myalgia was dose related. There was no significant myelosuppression, apart from a small and transient fall in platelet count. Six patients experienced acute but transient skin flushing, dyspnea, hypotension, and bradycardia, probably related to the bryostatin 1 vehicle. TNF-alpha and IL-6 were detected in plasma at 2 and 24 hours after **treatment**, respectively, and the levels were dose related ($P = .02$). Two patients with metastatic **malignant** melanoma had partial remission after three or four cycles of therapy; remission lasted 6 weeks and 10+ months, respectively. CONCLUSIONS: The dose-limiting toxicity of bryostatin 1 was myalgia. Plasma IL-6 and TNF-alpha concentrations were increased within 24 hours of therapy. Antitumor activity against **malignant** melanoma was observed early in the course of **treatment**. Implications: The recommended dosage of bryostatin 1 for phase II studies is 25 micrograms/m2 by intravenous infusion for 1 hour once a week for 3 weeks, with no **treatment** in the 4th week. IL-6 and TNF-alpha plasma concentrations may be useful in monitoring biological activity of bryostatin 1. Future studies should explore use of this drug with other conventional immune modulators and conventional cytotoxic drugs.

18/3,AB/80 (Item 80 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07843051 93373873 PMID: 7689954

Growth **factor** modulation of insulin-like growth **factor**
-binding proteins in rat osteoblast-like cells.

Chen T L; Chang L Y; DiGregorio D A; Perlman A J; Huang Y F
Department of Cell Analysis, Genentech, Inc., South San Francisco,
California 94080.

Endocrinology (UNITED STATES) Sep 1993, 133 (3) p1382-9,
ISSN 0013-7227 Journal Code: 0375040

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Insulin-like growth **factors** (IGFs) stimulate growth and differentiation of osteoblasts in culture, and these biological functions can be modulated by their binding proteins (IGFBPs). Previous studies have shown that IGFBP-2 is the major IGFBP synthesized by fetal rat osteoblast-like (ROB) cells, which also secrete a minor 24-kilodalton IGFBP, presumably IGFBP-4. In this study we examined the modulation of the abundance of IGFBPs by various growth **factors**. By immunoprecipitation and ligand blotting, we have proved that the 24 kilodalton protein, which appeared at 25 kilodalton in the present study, is IGFBP-4, whose level was strongly enhanced by basic fibroblast growth **factor** (bFGF) and platelet-derived growth **factor** BB homodimer. Induction of IGFBP-4 by these **factors** was detected at 1 nM (10- to 30-fold) and increased to 50- to 70-fold of control at 10 nM. The abundance of IGFBP-4 was also increased but to a lesser degree by transforming growth **factor**-alpha (TGF-alpha) and epidermal growth **factor** (EGF). As opposed to the actions of other growth **factors**, TGF-beta 1 substantially lowered the levels of IGFBP-2 and IGFBP-4. PTH and PTH-related peptide did not induce IGFBPs in ROB cells. This is in contrast to the findings from a **malignant** rat osteoblast-like cell line, UMR 106-01. Since the level of IGFBP-4 after bFGF **treatment** remained elevated in the presence of hydroxyurea, the induction was likely to be independent of the stimulatory effects of these **factors** on mitogenesis. To further examine the mechanisms by which IGFBPs were regulated, cultures were **treated** with actinomycin D and cycloheximide with and without bFGF. Although the synthesis of IGFBP-2 and IGFBP-4 were both inhibited by cycloheximide, actinomycin D blocked the synthesis of basal and bFGF-induced IGFBP-4 but not IGFBP-2. Total RNA extracted from ROB cells and hybridized with specific rat complementary DNAs for IGFBP-2 and IGFBP-4 showed single transcripts of 1.3 and 2.2 kilobases, respectively. Regardless of the changes at the protein level, the abundance of IGFBP-4 transcripts was not different from the vehicle-**treated** controls at 2, 8, and 24 h after bFGF **treatment**. Similarly, the levels of messenger RNA for IGFBP-2 and IGFBP-4 did not change during the same time course in the TGF-beta 1 **treatment**. These data demonstrate that in ROB cells, the abundance of IGFBPs is differentially regulated by various growth **factors**.
(ABSTRACT TRUNCATED AT 400 WORDS)

18/3,AB/81 (Item 81 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07830961 93364040 PMID: 1339190

Molecular analysis of the t(15;17) translocation in acute promyelocytic leukaemia.

Borrow J; Solomon E

Somatic Cell Genetics Laboratory, Imperial Cancer Research Fund, London, UK.

Bailliere's clinical haematology (ENGLAND) Oct 1992, 5 (4)
p833-56, ISSN 0950-3536 Journal Code: 8800474

Document type: Journal Article; Review; Review, Academic

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

APL (FAB M3) is a unique type of myeloid leukaemia characterized by specific clinical, morphological, cytogenetic and molecular features. An early and accurate diagnosis is necessary to initiate therapy and **treat** the life-threatening coagulopathy caused by release of procoagulants from the abundant promyelocytic granules. Cytogenetically the disease is characterized by a reciprocal translocation between the long arms of chromosomes 15 and 17, t(15;17)(q21;q22), which is seen in almost every patient with APL but in no other form of malignancy. The presence of this translocation, often as the only karyotypic change, suggests that potentially leukaemogenic sequences are located at the breakpoints and are activated by rearrangement. The recent cloning of the breakpoints by three groups has demonstrated that the retinoic acid receptor alpha gene (RARA) on chromosome 17 is fused to a previously undescribed **transcription factor** gene, PML, on chromosome 15. The DNA-binding motifs of both the RARA and PML proteins, together with the ligand-binding domain of RARA, are combined in a single fusion protein which may dysregulate either retinoic acid or PML-sensitive pathways. Identification of these dysregulated target genes has become the next molecular goal for research on APL. Intriguingly, some APLs not only express the PML-RARA fusion protein but also the reciprocal RARA-PML fusion protein, although the contribution of this product is unclear. The PML-RARA chimaeric protein is presumably the target during the striking differentiation therapy achieved with all-trans retinoic acid. This therapy induces the **malignant** promyelocytes to mature and die, rather than continue proliferating. Moreover, it represents the first direct connection between a genetic defect and clinical **treatment**. (ABSTRACT TRUNCATED AT 250 WORDS)

18/3,AB/82 (Item 82 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07823938 93356443 PMID: 8352523

Modulation of c-myc, c-myb, c-fos, c-sis and c-fms proto-oncogene expression and of CSF-1 transcripts and protein by phorbol diester in human **malignant** histiocytosis DEL cell line with 5q 35 break point.

Gogusev J; Barbey S; Nezelof C

Unite Inserm 90, Hospital Necker-Enfants Malades, Paris, France.

Anticancer research (GREECE) Jul-Aug 1993, 13 (4) p1043-7,

ISSN 0250-7005 Journal Code: 8102988

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Following exposure to phorbol ester (TPA), DEL cell line, a human **malignant** histiocytosis (MH) cell line, is able to differentiate along a macrophage phenotype and thus it provides a suitable model for analyzing the sequential and differential gene expression associated with monocyte/macrophage differentiation. C-myc, c-myb, c-fos, c-sis and c-fms expression were determined by Northern analysis at various times following TPA **treatment**. The results showed that TPA down-modulated the constitutive expression of c-myc, c-myb, and c-fms, mRNA to low but still detectable levels. Conversely, TPA-induced differentiation resulted in transient appearance of c-fos, whereas no change in the level of c-sis and actin transcripts were observed. Thus, the c-fms and c-sis genes appear to be regulated in a specific manner in this **malignant** histiocytosis derived cell line. Furthermore, these investigations demonstrated a constitutive CSF-1 gene expression which transiently increased at mRNA and also at protein level as evaluated by a murine bone marrow CFU bioassay.

Through this drug-induced modulation, the DEL cell line offers an additional model for studying some of the subtle interrelations existing between a growth **factor** (CSF-1) and its receptor (c-fms) in the monocyte/macrophage system.

18/3,AB/83 (Item 83 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07820522 93352540 PMID: 8349628

Cytokine regulation of low density lipoprotein receptor gene **transcription** in HepG2 cells.

Stopeck A T; Nicholson A C; Mancini F P; Hajjar D P
Department of Biochemistry, Cornell University Medical College, New York, New York 10021.

Journal of biological chemistry (UNITED STATES) Aug 15 1993, 268
(23) p17489-94, ISSN 0021-9258 Journal Code: 2985121R
Contract/Grant No.: HL-02738; HL; NHLBI; HL-46403; HL; NHLBI; RR-00085;
RR; NCRR

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Elevated plasma levels of cytokines have been demonstrated in inflammatory, **malignant**, and infectious diseases. These disease states are often associated with abnormal lipid metabolism and reductions in plasma cholesterol levels. To determine if inflammatory cytokines could influence hepatic lipid metabolism, we evaluated low density lipoprotein (LDL) receptor function and gene expression in cytokine stimulated HepG2 cells, a hepatoblastoma-derived cell line which shares many functional similarities with normal hepatocytes. Tumor necrosis **factor**-alpha (TNF) and interleukin-1 beta (IL-1) increased LDL binding to HepG2 cells in a dose-responsive manner. Other cytokines including macrophage-colony stimulating **factor**, granulocyte macrophage-colony stimulating **factor**, and gamma-interferon had no significant effects on LDL binding. Increased binding in response to TNF or IL-1 was paralleled by increased steady-state levels of LDL receptor mRNA. Evaluation of LDL receptor mRNA half-life revealed no significant change in mRNA stability between control and TNF- or IL-1-stimulated cells. A fusion gene construct consisting of 1563 base pairs of the 5'-flanking DNA of the human LDL receptor gene was coupled to a luciferase reporter gene, transfected into HepG2 cells, and promoter activity was assayed after TNF and IL-1 challenge to the cells. TNF and IL-1 increased promoter activity 200-400%, while **treatment** with LDL inhibited promoter activity by 70-85%. TNF or IL-1 co-incubation with LDL could not override transcriptional inhibition by LDL. Pretreatment with cycloheximide prevented induction of LDL receptor mRNA by TNF, but not by IL-1, suggesting stimulation of LDL receptor **transcription** by TNF requires protein synthesis. We propose that TNF and IL-1, acting via distinct signal transduction pathways, increase surface LDL receptors by increasing gene **transcription**. Our findings suggest that cytokine-induced hypocholesterolemia may be related to TNF and/or IL-1 stimulation of hepatic LDL receptor gene expression and function.

18/3,AB/84 (Item 84 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07815116 93346476 PMID: 8344985

Transforming growth **factor** beta 1 selectively regulates ornithine decarboxylase gene expression in **malignant** H-ras transformed fibrosarcoma cell lines.

Hurta R A; Greenberg A H; Wright J A

Manitoba Institute of Cell Biology, University of Manitoba, Winnipeg,

Canada.

Journal of cellular physiology (UNITED STATES) Aug 1993, 156

(2) p272-9, ISSN 0021-9541 Journal Code: 0050222

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Negative growth regulators such as the transforming growth factor beta (TGF-beta) family appear to be important inhibitors in most tissue types. However, inhibition of DNA synthesis and cell proliferation is frequently lost during **malignant** transformation, and in some cases, tumor cell proliferation is actually stimulated by TGF-beta. The present study demonstrates a novel link between alterations in TGF-beta regulation during **malignant** conversion, and the expression of ornithine decarboxylase, a key rate-limiting activity in the biosynthesis of polyamines, and an enzyme that plays an important role in cell growth and differentiation. A panel of radiation and H-ras transformed mouse 10T1/2 cell lines exhibiting increasing **malignant** potential was investigated for possible TGF-beta 1 mediated changes in ornithine decarboxylase gene expression. Selective induction of gene expression was observed since only H-ras transformed cell lines with **malignant** potential exhibited marked elevations in ornithine decarboxylase message levels. Ornithine decarboxylase gene expression in nontransformed 10T1/2 cells and cell lines capable of only benign tumor formation was unaffected by TGF-beta 1 **treatment**. H-ras transformed cells were transfected with a plasmid placing the TGF-beta 1 coding region under the control of a zinc sensitive metallothionein promoter. When these cells were cultured in the presence of zinc an elevation of TGF-beta 1 mRNA was observed within 30 min. This increase in TGF-beta 1 message closely coincided with an elevation in ornithine decarboxylase message, and preceded an induction of jun-B, an early response gene in cells sensitive to TGF-beta 1 stimulation. Evidence for regulation of ornithine decarboxylase gene expression by TGF-beta 1 at both **transcription** and posttranscription was found. Actinomycin D pretreatment of **malignant** cells prior to TGF-beta 1 exposure prevented the increase in ornithine decarboxylase message. Marked differences in the rates of ornithine decarboxylase message decay were observed when cells **treated** with TGF-beta 1 were compared to untreated controls, with the half-life of ornithine decarboxylase mRNA increasing from 2.5 h in untreated cells to 17.5 h in cells exposed to TGF-beta 1. In addition, evidence was obtained for a cycloheximide sensitive regulator of ornithine decarboxylase gene expression, since the presence of this protein synthesis inhibitor increased the levels of ornithine decarboxylase message, and this effect was synergistically augmented by exposure of cells to cycloheximide and induction of TGF-beta 1 gene expression together. (ABSTRACT TRUNCATED AT 400 WORDS)

18/3,AB/85 (Item 85 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07763313 93290264 PMID: 8512252

Molecular and biochemical reprogramming of oncogenesis through the activity of prooxidants and antioxidants.

Schwartz J L; Antoniades D Z; Zhao S

Department of Oral Pathology and Oral Medicine, Harvard School of Dental Medicine, Boston, Massachusetts 02115.

Annals of the New York Academy of Sciences (UNITED STATES) May 28 1993, 686 p262-78; discussion 278-9, ISSN 0077-8923

Journal Code: 7506858

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The antioxidant alpha-tocopherol and the weaker antioxidant and

prooxidant chemopreventative, beta-carotene have been shown to inhibit tumor cell growth in vivo and in vitro. In some epidemiologic studies their serum levels were demonstrated to be inversely related to the incidence of **malignant** tumor. We hypothesized two basic pathways triggered by antioxidants and prooxidants, which resulted in the control of tumor cell growth. These included changes in phosphorylation and ultimately **transcription**. Specifically, the prooxidant beta-carotene **treatment** produced an oxidative stress resulting in the selective induction of heat shock proteins (hsps). These proteins and other proteins that were possibly oxidized were associated with the increased expression of cyclins (A and D) and increased cdc2 kinase expression. An increase in expression of phosphoproteins, such as p53 (tumor suppressor form) was also discerned. The level of expression for the **transcription factor** c-fos was reduced. Growth **factors** that contribute to tumor cell growth were also reduced. Increased DNA fragmentation, depression of proliferation and intracellular calcium levels, the accumulation of tumor cells in G0-->G1, and morphologic changes, were consistent with programmed cell death. Antioxidants such as alpha-tocopherol bound to membrane-associated proteins could inhibit the development of peroxidation products (hydroxyl radicals (.OH)), which attack proteins and modify their function and promote their degradation. Some kinases such as, cdc2 may be increased in activity, which would explain the observed increased expression of tumor suppressor p53, the accumulation of the tumor cells in G1 of the cell cycle and the inhibition of tumor cell proliferation. A reduction in oxidant radicals could also reduce **transcription factor** products, such as c-myc. Indirectly this result may occur through changes in nuclear translocation (signaling) NF-AT or the Rel-related family of **transcription factors**, including NF-kB (p50 or p65) or inhibition of immunophilin-calmodulin activity. Although the data remains fragmentary there are common points for control for tumor cell growth resulting from the effects of alpha-tocopherol or beta-carotene **treatment**. These changes involve phosphorylation and protein expression. Ultimately there is a reduction of important **transcription factor** protein products, a reduction in response to growth **factors**, and suppression of cell proliferation, resulting in increased control of the cell cycle.

18/3,AB/86 (Item 86 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07762932 93289864 PMID: 8511989

Production of leukemia inhibitory **factor** mRNA and protein by **malignant** and immortalized bone cells.

Marusic A; Kalinowski J F; Jastrzebski S; Lorenzo J A

Veterans Administration Medical Center, Newington, Connecticut.

Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research (UNITED STATES) May 1993, 8 (5) p617-24, ISSN 0884-0431 Journal Code: 8610640

Contract/Grant No.: AR31263; AR; NIAMS; AR38933; AR; NIAMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Leukemia inhibitory **factor** (LIF) is a recently characterized glycoprotein with complex biologic activities on bone cells. We tested various rodent and human immortalized and **malignant** bone cell lines and primary osteoblast-enriched cell cultures from fetal rat calvarial digests for expression of LIF mRNA and LIF protein. Both human and rodent immortalized and **malignant** cells expressed a single 4.4 kb mRNA transcript that hybridized to a human LIF cDNA probe in Northern blots. LIF mRNA was undetectable in unstimulated rodent osteoblast-like cells lines MC3T3-E1 and Pyla. However, **treatment** with LPS (10 micrograms/ml), TGF-beta (1 ng/ml), TNF-alpha (100 ng/ml) or inhibitors of protein

synthesis (cycloheximide, emetine, puromycin, and anisomycin) induced the expression of LIF message in these cells. In contrast, primary osteoblast-enriched cells did not express LIF mRNA in Northern blot assays either constitutively or after **treatment** with TNF-alpha or cycloheximide. The human osteosarcoma cells lines U-2 OS and Saos-2 constitutively expressed LIF mRNA and did not respond to LPS **treatment**. However, phorbol myristate acetate (PMA), an activator of protein kinase C, was a potent stimulator of LIF message in Saos-2 but not U-2 OS cells. The effects of PMA (0.5 ng/ml) on LIF mRNA in Saos-2 cells were detectable at 1 h and maximal at 6 h. TNF-alpha (100 ng/ml) and inhibitors of protein synthesis also increased LIF mRNA in both Saos-2 and U-2 OS cells. LIF protein was also detected constitutively in the conditioned medium from both Saos and U-2 OS cells. (ABSTRACT TRUNCATED AT 250 WORDS)

18/3,AB/87 (Item 87 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07738038 93265465 PMID: 8495426

Sensitization of tumor cells to tumor necrosis **factor** action by the protein kinase inhibitor staurosporine.

Beyaert R; Vanhaesebroeck B; Heyninck K; Boone E; De Valck D; Schulze-Osthoff K; Haegeman G; Van Roy F; Fiers W

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Cancer research (UNITED STATES) Jun 1 1993, 53 (11) p2623-30,
ISSN 0008-5472 Journal Code: 2984705R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Tumor necrosis **factor** (TNF), first described as a cytokine with tumor-necrotizing activity, is now known to be a pleiotropic molecule. The molecular mechanisms responsible for the cytotoxic activity of TNF on **malignant** cells are still largely unknown. In this study, we report that the protein kinase inhibitor staurosporine (56 to 1500 nM) increases about 500 times the in vitro cytotoxic activity of TNF for several murine and human tumor cell lines. Even some tumor cell lines which are resistant to TNF cytotoxicity could be sensitized to TNF killing by staurosporine. In the L929 fibrosarcoma cell line, staurosporine also enhanced the transcriptional activation of interleukin 6 synthesis by TNF (500-fold stimulation at 56 nM). At the biochemical level, staurosporine increased the TNF-mediated activation of phospholipases C and D and the **transcription factor** NF-kappa B in L929 cells. The TNF-sensitizing effect of staurosporine does not seem to be mediated by one of the currently known staurosporine-sensitive kinases, as various other inhibitors which also inhibit one or more of these kinases were not synergistic with TNF. Interestingly, staurosporine (1 microgram) also enhanced the in vivo antitumor activity of TNF against a murine tumor model (L929 fibrosarcoma) in athymic nude mice (Swiss-nu/nu; s.c. **treatment**). These results suggest that TNF responsiveness of tumor cells is regulated by a novel staurosporine-sensitive target and that the combination of TNF and staurosporine may open new strategies of tumor **treatment**.

18/3,AB/88 (Item 88 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07698569 93223212 PMID: 8385577

Tumor necrosis **factor** alpha as an autocrine and paracrine growth **factor** for ovarian cancer: monokine induction of tumor cell proliferation and tumor necrosis **factor** alpha expression.

Wu S; Boyer C M; Whitaker R S; Berchuck A; Wiener J R; Weinberg J B; Bast

R C

Department of Medicine, Duke University Medical Center, Durham, North Carolina 27710.

Cancer research (UNITED STATES) Apr 15 1993, 53 (8) p1939-44,

ISSN 0008-5472 Journal Code: 2984705R

Contract/Grant No.: R01 CA39930; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Ovarian tumor cells produce macrophage colony stimulating **factor**, a potent chemoattractant for monocytes. Monocytes and macrophages produce tumor necrosis **factor** alpha (TNF-alpha) and interleukin 1 alpha or interleukin 1 beta (IL-1 beta) that can stimulate ovarian tumor cell growth. The present study has explored whether paracrine stimulation by monocyte derived cytokines might induce autocrine growth stimulation of normal and **malignant** ovarian epithelial cells. Endogenous expression of TNF-alpha mRNA was detected in ascites ovarian cancer cells isolated directly from patients, but not in established cultures of normal or **malignant** ovarian epithelial cells. When ascites tumor cells were cultured for 7 days, TNF-alpha expression ceased but could be reinduced by **treatment** with TNF-alpha or IL-1 beta. Ascites fluid contained concentrations of the cytokines that could mediate these effects. Similarly, **treatment** of normal or **malignant** ovarian epithelial cells with purified recombinant IL-1 beta or TNF-alpha induced **transcription** of TNF-alpha mRNA within 1 h. TNF-alpha protein could be detected by enzyme-linked immunosorbent assay in conditioned medium from IL-1 beta **treated** ovarian cancer cells. [3H]thymidine incorporation by normal or **malignant** ovarian epithelial cells was stimulated by a 24-h incubation with IL-1 beta or TNF-alpha. Stimulation of proliferation by IL-1 beta could be partially blocked by an antibody against TNF-alpha or by soluble TNF-alpha-receptor. Thus, TNF-alpha may function as both an autocrine and a paracrine growth **factor** in ovarian cancer.

18/3,AB/89 (Item 89 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07671020 93194748 PMID: 8449855

Acute promyelocytic leukemia. New insights into diagnosis and therapy.

Frankel S R

Department of Medicine, Roswell Park Cancer Institute, Buffalo, New York.

Hematology/oncology clinics of North America (ENGLAND) Feb 1993,

7 (1) p109-38, ISSN 0889-8588 Journal Code: 8709473

Document type: Journal Article; Review; Review, Academic

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The clinical and laboratory features of APL are distinct. APL has been effectively **treated** with anthracyclines. Postremission therapy and the addition of other cytotoxic agents in induction may be beneficial. Early deaths remain a problem despite improved management of coagulopathy. The cytogenetic marker, t(15;17), reflects a molecular defect that splices two **transcription factors**, PML and RARA, to produce chimeric mRNA and proteins. RA, the natural ligand for RARA, is able to induce CR by stimulating differentiation and maturation of the **malignant** cells. The addition of RA to the therapeutic armamentarium of the hematologic oncologist will allow further refinement of the management of these patients. Diagnosis is unambiguous because the molecular defect can be readily detected. Our understanding of the biology downstream of the affected genes is incomplete. Other retinoids may be more effective than all-trans RA and may avoid the fall in plasma levels seen in patients chronically **treated** with RA. Combination of retinoids with other cytokines or cytotoxic agents may decrease the immediate mortality and

improve long-term DFS in APL.

18/3,AB/90 (Item 90 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07669350 93192540 PMID: 8448375

Amplified genes in human gliomas.

Collins V P

Department of Pathology I, Sahlgrenska Hospital, Stockholm, Sweden.

Seminars in cancer biology (UNITED STATES) Feb 1993, 4 (1)

p27-32, ISSN 1044-579X Journal Code: 9010218

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The most common types of brain tumors in adults are collectively known as gliomas. The most common glioma is the most **malignant**, the glioblastoma. Double minute chromosomes, known to represent amplified genes, are found in 50% of glioblastomas. Four genes have been identified as being amplified in more than single cases of glioblastomas; MYCN, GLI, PDGFRA and EGFR. The first three have been reported in a few per cent of **malignant** gliomas, and EGFR in around 40% of glioblastomas. The latter two genes code for growth **factor** receptors. On amplification, the genes for these receptors frequently become rearranged, resulting in changes in the regions of their transcripts that code for the extra-cellular domains of these proteins. Such aberrant proteins may provide us with cell-surface, tumor-specific, epitopes. These findings provide simple examples of the impact the use of modern molecular biological techniques will have for our understanding and **treatment** of tumors in the future.

18/3,AB/91 (Item 91 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07666436 93188546 PMID: 8445950

All-trans retinoic acid induces monocyte growth **factor** receptor (c-fms) gene expression in HL-60 leukemia cells.

Hsu H C; Yang K; Kharbanda S; Clinton S; Datta R; Stone R M

Laboratory of Clinical Pharmacology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115.

Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K (ENGLAND) Mar 1993, 7 (3) p458-62, ISSN

0887-6924 Journal Code: 8704895

Contract/Grant No.: CA34283; CA; NCI; CA42802; CA; NCI; K08-CA-01352; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

All-trans-retinoic (ATRA) **treatment** of patients with acute promyelocytic leukemia results in differentiation of the **malignant** cells and a high complete remission rate. ATRA **treatment** induced granulocytic differentiation in HL-60 cells as assessed by nitroblue tetrazolium (NBT) reduction, but had no effect on non-specific esterase (NSE) straining, as expected in cells maturing along the monocytic lineage. However, our results demonstrate that ATRA (0.1-10 microM) induces expression of the c-fms (monocyte colony-stimulating **factor** receptor) gene in HL-60 cells. This effect was detectable after 2 days and expression was maximal at 5 days. Similar results were obtained during **treatment** with cis-retinoic acid (CRA), hexamethylene bisacetamide (HMBA), or dimethyl sulfoxide (DMSO). The results also demonstrate that ATRA-induced c-fms expression is potentiated by exposure to tumor necrosis **factor**

alpha (TNF alpha) or dibutyryl cyclic adenosine monophosphate (cAMP). The induction of c-fms transcripts by ATRA is associated with induction of M-CSF-binding ability, suggesting cell surface expression of the monocyte growth **factor** receptor. Our results indicate that retinoic acid can induce features of both monocytic and granulocytic differentiation in HL-60 cells.

18/3,AB/92 (Item 92 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07646239 93171605 PMID: 7679696

TSG-14, a tumor necrosis **factor**- and IL-1-inducible protein, is a novel member of the pentaxin family of acute phase proteins.

Lee G W; Lee T H; Vilcek J

Department of Microbiology, New York University Medical Center, NY 10016.

Journal of immunology (Baltimore, Md. : 1950) (UNITED STATES) Mar 1 1993, 150 (5) p1804-12, ISSN 0022-1767 Journal Code: 2985117R

Contract/Grant No.: R35CA49731; CA; NCI; T32AI07180; AI; NIAID

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

TNF-stimulated gene (TSG)-14 was originally identified as a TNF-inducible gene in a differentially screened cDNA library derived from TNF-**treated** normal human FS-4 fibroblasts. Analysis of the TSG-14 cDNA sequence revealed a major open reading frame encoding a protein of 381 amino acids, including a hydrophobic signal peptide sequence. The predicted protein shows 23 to 27% sequence homology to C-reactive protein and serum amyloid P-component, members of the pentaxin family of acute phase proteins. In addition, TSG-14 protein contains a sequence motif common among the pentaxin proteins. The ability of the TSG-14 cDNA to encode a protein of the correct molecular size was confirmed in a cell-free **transcription** /translation system. In vitro translation in the presence of microsomes confirmed that the protein has a cleavable signal peptide sequence, and that it is glycosylated. TSG-14 mRNA is rapidly elevated from almost undetectable levels in untreated FS-4 cells to high levels in cells **treated** with TNF or IL-1. A moderate increase in TSG-14 mRNA was observed in FS-4 cells **treated** with the glucocorticoid dexamethasone. Nuclear run-on analysis indicated that TNF induces the expression of the TSG-14 gene at the transcriptional level, and that de novo protein synthesis is not required for induction of TSG-14 mRNA. Expression of TSG-14 mRNA was also detected after exposure to TNF in vascular endothelial cells; however, little or not expression of TSG-14 message was observed in cell lines derived from **malignant** tumors. Our data strongly suggest that TSG-14 is a novel member of the pentaxin family of acute phase proteins.

18/3,AB/93 (Item 93 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07636761 93155092 PMID: 8381414

Tumor necrosis **factor** (TNF) up-regulates the expression of p75 but not p55 TNF receptors, and both receptors mediate, independently of each other, up-regulation of transforming growth **factor** alpha and epidermal growth **factor** receptor mRNA.

Kalthoff H; Roeder C; Brockhaus M; Thiele H G; Schmiegel W

Department of Immunology, University Hospital, Hamburg, Germany.

Journal of biological chemistry (UNITED STATES) Feb 5 1993, 268

(4) p2762-6, ISSN 0021-9258 Journal Code: 2985121R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The expression and cytokine-mediated regulation of the two different receptors for tumor necrosis factor, TNF-R-75 and TNF-R-55, was investigated in human **malignant** epithelial cell lines. Here we show that cells **treated** with TNF-alpha up-regulate the TNF-R-75 mRNA and protein levels. No changes were seen regarding the level of TNF-R-55 transcripts. Phospholipase and protein kinase C inhibitors abrogated the signal transduction pathway of TNF-mediated TNF-R-75 mRNA up-regulation which proceeded in the absence of transcriptional activation. This process was also elicited by an agonistic antibody binding specifically to TNF-R-55. Ligand binding assays using specific inhibitory antibodies showed a marked shift in active binding sites from p55 to p75 without significant changes in the total binding for TNF-alpha after up-regulation of p75 TNF-R. This ligand-induced regulation of one of the corresponding receptors has so far only been detected in **malignant** epithelial cells and not in hematopoietic cell lines. In our search for a specific function we were able to show that p75 is the specific receptor for TNF-mediated up-regulation of transforming growth factor alpha mRNA, whereas p55 is the signal transducer for TNF-induced up-regulation of epidermal growth factor receptor mRNA. This is the first demonstration of an exclusive function of TNF-R-75 in cells of epithelial origin.

18/3,AB/94 (Item 94 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07595348 93121542 PMID: 1362141

Role of estrogen receptor variants in the development of hormone resistance in breast cancer.

Sluyser M

Division of Tumor Biology, The Netherlands Cancer Institute, Amsterdam.
Clinical biochemistry (CANADA) Dec 1992, 25 (6) p407-14,

ISSN 0009-9120 Journal Code: 0133660

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Recent evidence suggests that the progression to hormone resistance in some breast tumors is due to mutations in the estrogen receptor (ER). Various types of ER variants have been found in breast cancer biopsies and breast cancer cell lines. The ER variants include dominant-positive receptors that are transcriptionally active in the absence of estrogen, and dominant-negative receptors that are themselves transcriptionally inactive but prevent the action of the normal receptor. The mechanisms by which these variants cause loss of hormonal control is becoming clear. ER variants may be prognostic **factors** for breast cancer. By modifying the action of ER variants, it should be possible to develop new strategies for **treatment** of **malignant** breast disease.

18/3,AB/95 (Item 95 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07520047 93046238 PMID: 1330303

Retinoic acid receptor alpha in acute promyelocytic leukaemia.
de The H; Dejean A

Unite de Recombinaison et Expression Genetique (INSERM U163), Institut Pasteur, Paris.

Cancer surveys (UNITED STATES) 1992, 14 p195-203, ISSN
0261-2429 Journal Code: 8218015

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Acute promyelocytic leukaemia has two highly specific particularities: a t(15;17) chromosomal translocation and the ability of a differentiation inducer all-trans-RA, to revert the **malignant** phenotype both in vitro and in vivo. Molecular characterization of the t(15;17) translocation has shown that it fuses a previously unknown zinc finger encoding gene, PML, to the RAR alpha, suggesting a link between the molecular mechanism of transformation and of RA dependent differentiation. The PML/RAR alpha fusion receptor--which is functionally altered--may block RA target genes, impair RA mediated differentiation and lead to transformation. Alternatively, or in addition, the PML transduction pathway may also be affected. Although it is clear that RA **treatment** must relieve APL cells from differentiation arrest, so far no model can satisfactorily account for this effect.

18/3,AB/96 (Item 96 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07478335 93004747 PMID: 1382708

Nitric oxide modulation of human leukemia cell differentiation and gene expression.

Magrinat G; Mason S N; Shami P J; Weinberg J B
Division of Hematology-Oncology, VA Medical Center, Durham, NC.
Blood (UNITED STATES) Oct 15 1992, 80 (8) p1880-4, ISSN
0006-4971 Journal Code: 7603509
Contract/Grant No.: P01 32682; PHS; P50 AR39162; AR; NIAMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Nitric oxide (NO) functions as an intercellular messenger molecule in such varied contexts as neurotransmission, immune regulation, and the control of vascular tone. We report that NO, delivered as purified gas or released from the pharmacologic NO donors sodium nitroprusside or 6-morpholino-sydnominine, caused monocytic differentiation of cells of the human myeloid leukemia cell line HL-60 and altered gene expression. The **treated** cells stopped proliferating, became spread and vacuolated, had increased expression of nonspecific esterase and the monocyte marker CD14, and displayed increased capacity to produce hydrogen peroxide. Furthermore, these **treated** cells had increased steady-state expression of messenger RNA (mRNA) for tumor necrosis **factor**-alpha (TNF-alpha) and interleukin-1 beta (IL-1 beta), but decreased expression of mRNA for the proto-oncogenes c-myc and c-myb. The increase in TNF-alpha and IL-1 beta mRNA levels was due (at least in part) to a new **transcription** of these specific mRNAs. NO elaborated in the bone marrow microenvironment may have a role in normal and **malignant** hematopoietic cell growth and differentiation.

18/3,AB/97 (Item 97 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07423153 92357026 PMID: 1323055

Phosphorothioate antisense oligonucleotides against basic fibroblast growth **factor** inhibit anchorage-dependent and anchorage-independent growth of a **malignant** glioblastoma cell line.

Murphy P R; Sato Y; Knee R S
Department of Physiology and Biophysics, Dalhousie University, Halifax, Nova Scotia, Canada.
Molecular endocrinology (Baltimore, Md.) (UNITED STATES) Jun 1992
, 6 (6) p877-84, ISSN 0888-8809 Journal Code: 8801431
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM

Record type: Completed

Basic fibroblast growth factor (bFGF) is a broad spectrum mitogen for many cells of neuroectodermal origin, including glial cells. The human **malignant** glioblastoma cell line U87-MG expresses high steady state levels of the bFGF mRNA and contains abundant stores of biologically active bFGF protein. In the present study we have examined the contribution of endogenous bFGF to the autocrine growth of these cells. Using reverse **transcription** -polymerase chain reaction, U87-MG cells were shown to express the mRNAs for both bFGF and the bFGF receptor, confirming the existence of the basic requirements for an autocrine loop. Addition of 5 microM bFGF-specific antisense oligonucleotide to U87-MG cultures significantly inhibited the growth rate of these cells within 48 h and blocked proliferation beyond 2 days. The corresponding bFGF-specific sense oligonucleotide did not significantly inhibit cell proliferation over the course of these experiments. Similarly, antisense oligonucleotides significantly inhibited colony formation in soft agar, while the sense sequence was without effect. Western blotting with antihuman bFGF revealed that U87-MG cells synthesize three isoforms of bFGF, approximately 18, 23, and 25 kilodaltons (kDa) in size. The 23- and 25-kDa isoforms together comprise approximately 80% of the total cellular stores of bFGF. Antisense **treatment** for 4 days reduced the abundance of the 23- and 25-kDa isoforms by 64-74%, but had little effect on the 18-kDa isoform. The inhibitory effect of the antisense oligonucleotides on anchorage-dependent proliferation was reversed by the addition of recombinant 18-kDa human bFGF. (ABSTRACT TRUNCATED AT 250 WORDS)

18/3,AB/98 (Item 98 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07407760 92343934 PMID: 1353322

Transduction of psychosocial stress into the neurobiology of recurrent affective disorder.

Post R M

Biological Psychiatry Branch, NIMH, Bethesda, MD 20892.

American journal of psychiatry (UNITED STATES) Aug 1992, 149

(8) p999-1010, ISSN 0002-953X Journal Code: 0370512

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Early clinical observations and recent systematic studies overwhelmingly document a greater role for psychosocial stressors in association with the first episode of major affective disorder than with subsequent episodes. The author postulates that both sensitization to stressors and episode sensitization occur and become encoded at the level of gene expression. In particular, stressors and the biochemical concomitants of the episodes themselves can induce the protooncogene c-fos and related **transcription factors**, which then affect the expression of transmitters, receptors, and neuropeptides that alter responsivity in a long-lasting fashion. Thus, both stressors and episodes may leave residual traces and vulnerabilities to further occurrences of affective illness. These data and concepts suggest that the biochemical and anatomical substrates underlying the affective disorders evolve over time as a function of recurrences, as does pharmacological responsivity. This formulation highlights the critical importance of early intervention in the illness in order to prevent **malignant** transformation to rapid cycling, spontaneous episodes, and refractoriness to drug **treatment**.

18/3,AB/99 (Item 99 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07398750 92333174 PMID: 1629632

Enhanced production of plasminogen activator activity in human and murine keratinocytes by transforming growth factor-beta 1.

Keski-Oja J; Koli K

Department of Virology, University of Helsinki, Finland.

Journal of investigative dermatology (UNITED STATES) Aug 1992,
99 (2) p193-200, ISSN 0022-202X Journal Code: 0426720

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Transforming growth factor-beta (TGF beta) is the most potent known inhibitor of keratinocyte growth. Pericellular proteolytic activity is usually high in proliferating and **malignant** cells and decreased in resting or growth-arrested cells. We have therefore analyzed the effects of TGF beta 1 on the production of plasminogen activator activity by normal human keratinocytes and a mouse keratinocyte cell line under serum-free conditions. The plasminogen activator activity of the culture medium was analyzed using caseinolysis-in-agar and zymography assays, immunoblotting, and Northern hybridization analysis for the plasminogen activators (PA) and PA inhibitor-1 (PAI-1). Alterations of radiolabeled polypeptides were observed in fluorograms of gels. It was found that like in human epidermoid carcinoma cells picomolar concentrations of TGF beta 1 (0.2-20 ng/ml) enhanced total plasminogen activator activity in both keratinocyte cell systems. Zymographic and immunoblotting analyses of the medium indicated that the activator was of the urokinase type (u-PA). Immunoprecipitation and Concanavalin A affinity chromatography of the culture medium indicated that the cells also started to produce PAI-1. Analysis of the pericellular matrix preparations of the keratinocytes showed that PAI-1 is deposited to the pericellular space. Evidently due to elevated u-PA activity PAI-1 was removed from the extracellular matrix more rapidly in TGF beta 1-**treated** cells than from control cultures. Northern hybridization analysis of human keratinocytes showed that TGF beta 1 rapidly elevated both u-PA and PAI-1 mRNA levels. Comparison of the temporal induction profiles indicated that the mRNA for u-PA increased more slowly but was more persistent than that of PAI-1. Actinomycin D inhibited the induction of both u-PA and PAI-1 mRNA, suggesting that the induction was due to increased **transcription**. The results suggest that enhanced plasminogen activator activity can be associated with growth inhibition also in nonmalignant cells like cultured human or murine keratinocytes.

18/3,AB/100 (Item 100 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07298242 92225074 PMID: 1314190

Hydrocortisone-induced increase of PDGF beta-receptor expression in a human **malignant** mesothelioma cell line.

Versnel M A; Bouts M J; Langerak A W; van der Kwast T H; Hoogsteden H C; Hagemeijer A; Heldin C H

Department of Immunology, Erasmus University, Rotterdam, The Netherlands.

Experimental cell research (UNITED STATES) May 1992, 200 (1)
p83-8, ISSN 0014-4827 Journal Code: 0373226

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The effect of hydrocortisone (HC) on PDGF beta-receptor expression was studied in the human **malignant** mesothelioma cell line Mero-14. HC was found to induce a time- and dose-dependent increase of PDGF beta-receptor mRNA. Nuclear run off analysis revealed that HC induced increased **transcription** of the PDGF beta-receptor gene. The expression of PDGF beta-receptor protein was also elevated by HC as demonstrated with an immunoblotting assay. However, the number of PDGF-BB binding sites on the cell surface of Mero-14 remained unchanged upon HC **treatment**. These

results suggest that steroid hormones can regulate PDGF receptor expression in vivo.

18/3,AB/101 (Item 101 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07288432 92223373 PMID: 1562723

Acute- and chronic-phase chronic myelogenous leukemia colony-forming units are highly sensitive to the growth inhibitory effects of c-myb antisense oligodeoxynucleotides.

Ratajczak M Z; Hijiya N; Catani L; DeRiel K; Luger S M; McGlave P; Gewirtz A M

Department of Pathology, University of Pennsylvania School of Medicine, Philadelphia 19104.

Blood (UNITED STATES) Apr 15 1992, 79 (8) p1956-61, ISSN 0006-4971 Journal Code: 7603509

Contract/Grant No.: CA01324; CA; NCI; CA36896; CA; NCI; CA54384; CA; NCI;

+

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

We have previously demonstrated that **malignant** hematopoietic colony-forming units (CFUs) may be purged from normal CFU by exposure to c-myb antisense oligodeoxynucleotides (oligomers). This novel strategy appeared particularly promising for patients with chronic myelogenous leukemia (CML) in blast crisis, since in some cases complete elimination of bcr-abl-expressing cells was accomplished. We have examined 11 additional patients, including seven in chronic phase, in order to extend these initial observations. We sought in particular to determine if elimination of bcr-abl-expressing clones was a usual event. Exposure of CML cells to c-myb antisense oligomers resulted in inhibition of CFU-granulocyte, macrophage (CFU-GM)-derived colony formation in eight of 11 (73%) cases evaluated. Inhibition was antisense sequence-specific, dose-dependent, ranged between 58% and 93%, and was statistically significant (P less than or equal to .03) in seven of the eight cases. In two cases, CFU-granulocyte, erythrocyte, monocyte, megakaryocyte (CFU-GEMM)-derived colony formation was also examined and found to be inhibited by the c-myb antisense oligomers in a sequence-specific manner. To determine whether CML CFU had been reduced or eliminated after exposure to the antisense oligomers, we examined cells in the residual colonies for bcr-abl mRNA expression using a reverse **transcription**-polymerase chain reaction detection technique (RT-PCR). Eight cases were evaluated and in each case where antisense myb inhibited growth, bcr-abl expression as detected by RT-PCR was either greatly decreased or nondetectable. No residual leukemic CFU were demonstrable on replating of **treated** cells. These results suggest that c-myb antisense oligomers substantially inhibit the growth and survival of CML CFU in both chronic and blast phase of disease. They may therefore prove useful for both ex vivo and in vivo **treatment** of CML.

18/3,AB/102 (Item 102 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

06969583 91278914 PMID: 1905388

Tumor necrosis **factor** and c-fos expression in human peripheral-blood monocytes: expression is dependent on stage of in vitro differentiation.

Turpin J A; Blick M; Hester J P; Lopez-Berestein G

Department of Clinical Immunology and Biological Therapy, University of Texas M.D. Anderson Cancer Center, Houston.

Natural immunity and cell growth regulation (SWITZERLAND) 1991,
10 (1) p19-31, ISSN 0254-7600 Journal Code: 8407979

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The differentiation of monocytes and macrophages both in vitro and in vivo can be characterized by the modulation of specific functional and molecular phenotypes. We have determined in human peripheral-blood monocytes (HPBM) the role of in vitro differentiation on the expression of nonspecific tumoricidal activity, induction of soluble tumor necrosis factor (TNF) activity and TNF-specific mRNA **transcription**. HPBM were activatable by bacterial lipopolysaccharide (LPS; 1 microgram/ml) for nonspecific cytotoxicity to A375M (human **malignant** melanoma cell line) only during the first 24 h of in vitro differentiation. Activated supernatants of HPBM were found to be partially neutralizable (75 +/- 7%) by rabbit polyclonal anti-TNF antibody and, in freshly isolated HPBM, the release of soluble TNF activity determined by the L929 assay was found to occur only after activation with LPS. Maximal TNF release occurred at 8 h of LPS stimulation, and required both protein and RNA synthesis as evidenced by the ability of both actinomycin D and cycloheximide to inhibit its release. Neither control untreated HPBM nor recombinant interferon-gamma (rIFN-gamma; 1 U/ml)-**treated** HPBM alone released soluble TNF activity. Further in vitro culture determined that HPBM were activatable for TNF release out to 72 h of culture after which HPBM became resistant to LPS-mediated TNF release. The expression of TNF and c-fos mRNA was also determined during in vitro differentiation. Both TNF and c-fos mRNA were expressed in freshly isolated HPBM, and returned to baseline by 24 h of in vitro culture. **Treatment** of HPBM with LPS induced TNF **transcription** as late as 5 days of in vitro culture with maximal induction occurring during the first 48 h. rIFN-gamma significantly induced TNF **transcription** at 24 h in the absence of soluble TNF activity, but did not increase **transcription** at later times. The expression of nonspecific cytolytic activity, the release of soluble bioactive TNF and the induction of TNF and c-fos mRNA are regulated in HPBM by differentiation-determined processes.

18/3,AB/103 (Item 103 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

06914800 91224084 PMID: 1709093

Extinction of the HPV18 upstream regulatory region in cervical carcinoma cells after fusion with non-tumorigenic human keratinocytes under non-selective conditions.

Rosl F; Achtstatter T; Bauknecht T; Hutter K J; Futterman G; zur Hausen H
Institut fur Angewandte Tumorstudiologie, Deutsches Krebsforschungszentrum, Heidelberg, FRG.

EMBO journal (ENGLAND) Jun 1991, 10 (6) p1337-45, ISSN 0261-4189 Journal Code: 8208664

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Universal fuser' clones of a human papillomavirus type 16 positive cervical carcinoma cell line (SiHa) were established to study the effect of a non-tumorigenic fusion partner on the regulation of a stably integrated chloramphenicol acetyltransferase (CAT) gene controlled by the HPV18 upstream regulatory region under non-selective conditions. The CAT expressing cells were fused with both non-tumorigenic, spontaneously immortalized human keratinocytes (HaCaT) and non-modified SiHa cells. The resulting hybrids were characterized by restriction enzyme fragment length polymorphism analysis and flow cytometry. While the non-selectable, HPV18-driven indicator gene is constitutively expressed in SiHa cells, the CAT activity is extinguished in SiHa x HaCaT cells, but still present in SiHa x SiHa hybrids. Examination of the cytokeratin expression pattern

reveals that the keratinocyte phenotype seems not only to be dominant in terms of the extinction of the HPV18 regulatory region but also by the conservation of most of the differentiation markers of the non-tumorigenic fusion partner. Cycloheximide **treatment** and intracellular competition experiments using the transient COS7 fusion-amplification technique are accompanied by the reactivation of the marker gene in previously CAT- SiHa x HaCaT hybrids. These data strongly suggest that trans-acting negative regulatory **factors** derived from the non-malignant human keratinocytes are responsible for the extinction phenomenon.

18/3,AB/104 (Item 104 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

06865013 91175484 PMID: 1848774

Downstream sequences mediate induction of the mouse cathepsin L promoter by phorbol esters.

Troen B R; Chauhan S S; Ray D; Gottesman M M
Laboratory of Cell Biology, National Cancer Institute, NIH, Bethesda, Maryland 20892.

Cell growth & differentiation : the molecular biology journal of the American Association for Cancer Research (UNITED STATES) Jan 1991,

2 (1) p23-31, ISSN 1044-9523 Journal Code: 9100024

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The major excreted protein (MEP) of mouse fibroblasts is the precursor to a lysosomal acid protease (cathepsin L) whose synthesis is induced by **malignant** transformation, growth **factors**, tumor promoters, and cyclic AMP. We have previously cloned a functional gene for MEP from NIH 3T3 cells. When subcloned into chloramphenicol acetyl transferase (CAT) expression vectors, both 4-kilobase and 300 base pair fragments in the 5'-flanking region of the MEP gene confer CAT activity that is stimulated by cyclic AMP **treatment** but is not stimulated by phorbol ester **treatment** of NIH 3T3 cells. These fragments confer constitutive promoter activity that is comparable to that of the SV40 promoter. Primer extension, using RNA from cells transiently transfected with MEP-CAT fusion plasmids, demonstrates that phorbol ester **treatment** increases the amount of transcript from constructs containing both the promoter and sequences downstream of the **transcription** initiation site, including the first three introns, but not from constructs containing only the 5'-flanking region of the MEP gene. Nuclear run-off experiments confirm that the increase in endogenous MEP mRNA is mediated by increased **transcription** and not via relief of transcriptional attenuation. Since both the MEP promoter, which contains three potential binding sites for the AP-2 **transcription factor**, and the SV40 promoter, which contains both AP-1 and AP-2 binding sites, fail to respond to 12-O-tetradecanoylphorbol-13-acetate in NIH 3T3 cells, these upstream motifs are not sufficient to confer phorbol ester responsiveness in NIH 3T3 cells. These results suggest that the MEP gene is regulated in a complex manner by sequences both upstream and downstream of the **transcription** initiation site.

18/3,AB/105 (Item 105 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

06734325 91046024 PMID: 2172988

Introduction of nerve growth **factor** (NGF) receptors into a medulloblastoma cell line results in expression of high- and low-affinity NGF receptors but not NGF-mediated differentiation.

Pleasure S J; Reddy U R; Venkatakrishnan G; Roy A K; Chen J; Ross A H; Trojanowski J Q; Pleasure D E; Lee V M

Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia 19104.

Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Nov 1990, 87 (21) p8496-500, ISSN 0027-8424 Journal Code: 7505876

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Expression of the cloned human nerve growth **factor** receptor (NGFR) cDNA in cell lines can generate both high- and low-affinity binding sites. Since the inability to respond appropriately to differentiation **factors** such as NGF may contribute to determining the **malignant** phenotype of neuroblastomas, we sought to determine whether the same is true of medulloblastomas. To generate a human central nervous system neuronal cell line that would respond to NGF, we infected the medulloblastoma cell line D283 MED with a defective retrovirus carrying the cDNA coding for the human NGFR. The resultant cells (MED-NGFR) expressed abundant low- and high-affinity NGFRs, and NGF **treatment** induced a rapid transient increase of c-fos mRNA in the NGFR-expressing cells but not in the parent line or in cells infected with virus lacking the cDNA insert. However, the MED-NGFR cells did not internalize the NGFR at high efficiency, nor did they differentiate in response to NGF. Three important conclusions emerge from this study: (i) internalization of NGFRs is not necessary for some early rapid transcriptional effects of NGF; (ii) an unknown **factor** (s) that cooperates with the cloned NGFR in allowing high-affinity NGF binding is found in a primitive central nervous system cell line; and (iii) NGFRs introduced into and expressed by D283 MED (i.e., MED-NGFR) cells are partially functional but are unable to induce differentiation in these primitive neuron-like tumor cells, implying that high-efficiency receptor-mediated endocytosis of NGF and its receptor may be a necessary step in the cascade of events leading to NGF-mediated differentiation.

18/3,AB/106 (Item 106 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

06501146 90206596 PMID: 2157176

Transforming growth **factor**-beta represses **transcription** of the mouse mammary tumour virus DNA in cultured mouse mammary cells.

Cato A C; Mink S; Nierlich B; Ponta H; Schaap D; Schuurin E; Sonnenberg A

Kernforschungszentrum Karlsruhe, Institut für Genetik und Toxikologie, Federal Republic of Germany.

Oncogene (ENGLAND) Jan 1990, 5 (1) p103-10, ISSN 0950-9232
Journal Code: 8711562

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Increased expression of mouse mammary tumour virus (MMTV) is associated with hyperplastic alveolar growth and subsequent development of mammary cancers in the mouse. The expression of this virus is repressed when mammary tumour cells undergo sarcomatous transformation. We have demonstrated that a spontaneous progression of mouse mammary adenocarcinoma cells into highly **malignant** cells with the transformed phenotype is accompanied by an increased expression of transforming growth **factors** alpha and beta (TGF alpha and TGF beta), as well as a decreased expression of MMTV. Mouse mammary adenocarcinoma cells transformed with activated ras oncogene also expressed high levels of the transforming growth **factors** and a low level of MMTV. Thus a reverse correlation exists between the increased expression of the transforming growth **factors** and a low level of expression of MMTV. Mouse mammary cells that express

high levels of MMTV when **treated** with exogenous TGF alpha and TGF beta 1 showed a down regulation of MMTV expression in response to TGF beta 1 but not to TGF alpha. These results demonstrate that the repression of MMTV expression in mouse mammary tumour progression may be due in part to an increased expression of TGF beta.

18/3,AB/107 (Item 107 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06309532 90002958 PMID: 2571413

Gene activity during the early phase of androgen-stimulated rat prostate regrowth.

Katz A E; Benson M C; Wise G J; Olsson C A; Bandyk M G; Sawczuk I S; Tomashefsky P; Buttyan R
Department of Urology, Maimonides Medical Center, Brooklyn, New York 11219.

Cancer research (UNITED STATES) Nov 1 1989, 49 (21) p5889-94,

ISSN 0008-5472 Journal Code: 2984705R

Contract/Grant No.: CA47848; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Androgenic steroids regulate the proliferation rate of normal and **malignant** prostate cells. In order to investigate the molecular basis of this control, we utilized Northern and Western blot techniques to measure changes in the expression of individual genes during the early phase of prostate regrowth. Vestigial ventral prostate glands of 7-day castrated rats showed increased numbers of replicating cells within 12 h of continuous pharmacological testosterone replacement as demonstrated by flow cytometric procedures. The period prior to the onset of proliferative enhancement was accompanied by the sequential induction of a variety of transcripts encoding gene products often associated with cell growth. Within 1 h of **treatment**, mature mRNA transcripts for c-fos were induced 6-fold, returning to control levels by 2 h. Other genes showed transiently elevated transcript levels after 2 h (c-Ha-ras, c-Ki-ras) or after 8 h (c-myc, c-myb, beta-actin, and Mr 70,000 heat shock protein) of testosterone replacement. Expression of the polypeptide encoded by c-Ha-ras was coordinately enhanced (2-fold) during this period, but not to the levels of the transcript (20-fold induction). Transcripts encoding basic fibroblast growth **factor** were increased 16 h and later, subsequent to the earlier enhancement in the proliferation rate. By placing these genes in a defined temporal order with regard to their expression in response to testosterone, we have constructed a map of gene activity during early prostate regrowth. This map shows a number of genes, the products of which might participate in the mechanism by which androgens induce mitogenesis of prostate cells.

18/3,AB/108 (Item 108 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05949574 89034199 PMID: 2846555

Transforming growth **factor** beta 1 and cAMP inhibit **transcription** of epidermal growth **factor**- and oncogene-induced transin RNA.

Kerr L D; Olashaw N E; Matrisian L M
Department of Cell Biology, School of Medicine, Vanderbilt University, Nashville, Tennessee 37232.

Journal of biological chemistry (UNITED STATES) Nov 15 1988, 263

(32) p16999-7005, ISSN 0021-9258 Journal Code: 2985121R

Contract/Grant No.: CA09592; CA; NCI; HD05797; HD; NICHD; R01CA46845; CA;

NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Transin mRNA encodes a secreted metalloprotease which is transcriptionally induced in Rat-1 cells by epidermal growth factor (EGF) and a number of oncogenes. A role for transin in tumor progression is suggested by its overexpression in **malignant** and metastatic tumors compared to their benign counterparts. In an effort to elucidate mechanisms by which elevated transin expression may be inhibited, it has been determined that both transforming growth factor type beta 1 (TGF beta 1) and increased levels of intracellular cyclic 5'-adenosine monophosphate (cAMP) inhibit EGF and oncogene induction of transin mRNA. The inhibition of transin mRNA occurred at the level of **transcription** as demonstrated by nuclear run-on assays. EGF binding studies in Rat-1 cells showed no significant effect of cAMP or TGF beta 1 on EGF receptor number or affinity. We have also examined the effects of cAMP and TGF beta 1 on oncogene-induced transin using Rat-1 cells transformed by temperature-sensitive mutants of v-src and K-ras oncogenes. Both inhibitors prevented the induction of transin RNA as well as decreased the levels of transin once elevated at the permissive temperature. Despite the similarities in the actions of TGF beta 1 and cAMP on transin gene expression, TGF beta 1 **treatment** did not significantly elevate intracellular cAMP levels, thus making it unlikely that cAMP is a second messenger system for TGF beta 1 action. These studies suggest that the inhibitory effects of cAMP and TGF beta 1 occur by distinct pathways at the level of gene regulation.

18/3,AB/109 (Item 109 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

05928362 89008866 PMID: 2844850

Expression of platelet-derived growth factor (PDGF)-related transcripts and synthesis of biologically active PDGF-like proteins by human **malignant** epithelial cell lines.

Sariban E; Sitaras N M; Antoniadis H N; Kufe D W; Pantazis P

Laboratory of Clinical Pharmacology, Dana-Farber Cancer Institute, Boston, MA 02115.

Journal of clinical investigation (UNITED STATES) Oct 1988, 82

(4) p1157-64, ISSN 0021-9738 Journal Code: 7802877

Contract/Grant No.: CA-30101; CA; NCI; CA-38784; CA; NCI; HL-29583; HL; NHLBI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Human **malignant** epithelial cell lines were analyzed for expression of platelet-derived growth factor (PDGF) genes. Of the 12 cell lines tested, 9, derived from breast, lung, gastric, and ovarian carcinomas, were found to express both PDGF-1 and PDGF-2 genes. The levels of both PDGF-1 and PDGF-2 transcripts were superinduced when these cells were **treated** with cycloheximide, an inhibitor of protein synthesis. These cells also released an activity that in studies with BALB-c/3T3 cells, inhibited binding of 125I-labeled PDGF and stimulated incorporation of [3H]thymidine. This stimulating activity was inhibited after reduction of the conditioned media by mercaptoethanol or after preincubation with antibodies to PDGF. Moreover, this activity was not affected by heat **treatment**. Immunoprecipitation studies revealed that breast, lung, and gastric carcinoma cells produced PDGF-like proteins that migrated as 30- and 32-kD species under nonreducing conditions and as 15- and 16-kD species under reducing conditions. In contrast, **malignant** cells of ovarian origin produced 14-16-kD PDGF-like proteins that were unchanged in mobility after reduction. As PDGF receptors were not detected on these

malignant epithelial cells, the production of PDGF-like proteins may affect other cells in the microenvironment by paracrine mechanisms and may contribute to excessive cell proliferation, inflammatory reactions, and connective tissue remodeling seen in certain carcinomas.

18/3,AB/110 (Item 110 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

05924537 89028335 PMID: 3263191

In vitro and in vivo activation of B-lymphocytes: a flow cytometric study of chromatin structure employing 7-aminoactinomycin D.

Stokke T; Holte H; Steen H B

Department of Biophysics, Norwegian Radium Hospital, Oslo.

Cancer research (UNITED STATES) Dec 1 1988, 48 (23) p6708-14,

ISSN 0008-5472 Journal Code: 2984705R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The chromatin structure of a diploid precursor B-cell line (REH), in vitro-stimulated normal B-lymphocytes, and reactive and **malignant** lymph node B-lymphocytes was studied by staining formaldehyde-fixed, permeabilized cells with the DNA-specific fluorophore 7-aminoactinomycin D (7-AMD) and measuring single-cell fluorescence by flow cytometry. Resting peripheral blood B- and T-lymphocytes (G0 cells) bound low amounts of 7-AMD (7-AMD- phenotype), while G1 REH cells and purified B-cells stimulated with anti-mu + B-cell growth **factor** bound nearly twice as much 7-AMD (7-AMD+ phenotype). 7-AMD binding increased up to threefold and the differences in binding between G0 and G1 cells were nearly abolished when nuclei were isolated prior to fixation or when fixed whole cells were **treated** with DNase 1. 7-AMD binding increased in parallel with autofluorescence and approximately linearly with time during the G0-G1 transition of in vitro stimulated B-cells, as was determined by simultaneous measurements of 7-AMD fluorescence and autofluorescence or fluorescence of fluorescein isothiocyanate-labeled antibodies to the early activation antigen 4F2 and to the transferrin receptor. In cell suspensions from lymph node biopsies, the 7-AMD+ phenotype was a property of tumor cells in patients with high grade non-Hodgkin's lymphoma (H-NHL, Kiel classification, 5/5); cells with this phenotype were only found in one of nine low grade non-Hodgkin's lymphoma samples (L-NHL, 1/9). The other (8/9) L-NHL samples and the reactive lymph node contained only 7-AMD- cells. All tumors were diploid. The correlation observed between 7-AMD binding and DNase 1 susceptibility of DNA in chromatin (P less than 0.001) suggests that 7-AMD binding is a marker of general transcriptional activity. Surprisingly, the percentage of tumor cells in S phase did not correlate significantly with 7-AMD stainability (P = 0.07), while the light scattering (cell size) of G0/G1 cells was highly correlated to 7-AMD binding (P less than 0.001).

18/3,AB/111 (Item 111 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

05870183 88302158 PMID: 3043179

Transcriptional modulation of transin gene expression by epidermal growth **factor** and transforming growth **factor** beta.

Machida C M; Muldoon L L; Rodland K D; Magun B E

Department of Cell Biology and Anatomy, Oregon Health Sciences University, Portland 97201.

Molecular and cellular biology (UNITED STATES) Jun 1988, 8 (6)

p2479-83, ISSN 0270-7306 Journal Code: 8109087

Contract/Grant No.: CA-39181; CA; NCI; CA-39360; CA; NCI; HDO 7133-09; HD
; NICHD

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Transin is a transformation-associated gene which is expressed constitutively in rat fibroblasts transformed by a variety of oncogenes and in **malignant** mouse skin carcinomas but not benign papillomas or normal skin. It has been demonstrated that, in nontransformed Rat-1 cells, transin RNA expression is modulated positively by epidermal growth **factor** (EGF) and negatively by transforming growth **factor** beta (TGF-beta); other peptide growth **factors** were found to have no effect on transin expression. Results presented here indicate that both protein synthesis and continuous occupancy of the EGF receptor by EGF were required for sustained induction of transin RNA. **Treatment** with TGF-beta inhibited the ability of EGF to induce transin, whether assayed at the transcriptional level by nuclear run-on analysis or at the level of transin RNA accumulation by Northern (RNA) blot analysis of cellular RNA. TGF-beta both blocked initial induction of transin **transcription** by EGF and halted established production of transin transcripts during prolonged **treatment**. These results suggest that TGF-beta acts at the transcriptional level to antagonize EGF-mediated induction of transin gene expression.

18/3,AB/112 (Item 112 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

05628639 88055283 PMID: 3119348

Biological properties of suppressive E-receptor **factor** on lymphokine function.

Oh S K; Leung M F; Knee T; Williams J M

Department of Microbiology, Boston University School of Medicine, MA 02118.

European journal of immunology (GERMANY, WEST) Oct 1987, 17
(10) p1403-9, ISSN 0014-2980 Journal Code: 1273201

Contract/Grant No.: AM 33921; AM; NIADDK; CA 15129; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

A potent immunosuppressive **factor** isolated from **malignant** ascites fluids showed serological cross-reactivity with the E-receptor of human peripheral blood T lymphocytes. Thus, this **factor** was named suppressive E-receptor (SER) **factor**. In this study, we examined the effect of this immunosuppressor, SER, on lymphokine functions of human mononuclear cells participating in polyclonal T cell activation. SER is active at nanomolar concentrations in vitro and the inhibitory effect of SER was most pronounced when added at the initiation of stimulation with phytohemagglutinin or anti-T3 antibody. Concomitant with the inhibition on PHA-induced DNA synthesis, lymphocytes that were **treated** with SER failed to progress beyond G1 phase of cell cycle. These growth-arrested cells did expire after 7 days of culture in vitro. This anti-proliferative effect of SER was more easily demonstrated with normal lymphoid cells in culture than transformed cells or fibroblast cells. SER effectively interfered with the lympho-proliferative properties of interleukin 2 (IL 2) on human peripheral blood mononuclear cells and an IL 2-dependent murine cytotoxic T cell line. However, excess quantities of exogenous IL 2, especially when added in conjunction with IL 1, were able to partially overcome the ability of SER to inhibit T cell proliferation. In contrast to the inhibition on DNA synthesis of human lymphoblasts, expression of IL 2 receptor was only minimally inhibited by SER during the first 24 h of culture (24% inhibition at 12 h and 34% inhibition at 24 h) but it was followed by full expression of IL 2 receptor by 48 h. Thus, SER merely reduced the rate of expression of IL 2 receptor and was not able to inhibit

the **transcription** of new message from activated T lymphocytes. Taken together, these studies indicate that SER acts as a noncytolytic anti-proliferative **factor** on immune responses that are mediated by T cells. SER appears to act on a relatively late event during T cell activation, perhaps on some portion of the DNA replication pathway.

18/3,AB/113 (Item 113 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

05569029 87322693 PMID: 2888269

The interleukin-2 receptor on normal and **malignant** lymphocytes.
Waldmann T A

Advances in experimental medicine and biology (UNITED STATES)
1987, 213 p129-37, ISSN 0065-2598 Journal Code: 0121103

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Interleukin-2 (IL-2) is a lymphokine synthesized by T cells following activation. Resting T cells do not express IL-2 receptors, but receptors are rapidly expressed on T cells following interaction of the antigen-specific T-cell receptor complex with appropriately processed and presented antigens. Anti-Tac, a monoclonal antibody that recognized the IL-2 receptor, has been used to purify the receptor. The recognized the IL-2 receptor, has been used to purify the receptor. The receptor is a 55-Kd glycoprotein comprised of 272 amino acids including a single 19-amino transmembrane domain and a short intracytoplasmic domain composed of 13 amino acids at the carboxy terminus. Normal resting T cells and most leukemic T-cell populations examined did not express IL-2 receptors; however, the leukemic cells of all patients with human T-cell lymphotropic virus (HTLV-I)-associated adult T-cell leukemia (ATL) expressed the Tac antigen. In HTLV-I-infected cells, the 42-Kd long open reading frame (tat) protein encoded in part by the tat region of HTLV-I may act as a transacting activator that induces **transcription** of the IL-2 receptor gene, thus providing an explanation for the constant association of HTLV-I infection of lymphoid cells and IL-2 receptor expression. The constant display of large numbers of IL-2 receptors which may be aberrant in the ATL cells may play a role in the uncontrolled growth of these leukemic T cells. Patients with the Tac-positive ATL are being **treated** with both unmodified and toxin-conjugated forms of anti-Tac monoclonal antibody directed toward this growth **factor** receptor.

18/3,AB/114 (Item 114 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

05255606 87002626 PMID: 3019574

The interleukin-2 receptor on **malignant** cells: a target for diagnosis and therapy.

Waldmann T A

Cellular immunology (UNITED STATES) Apr 15 1986, 99 (1) p53-60

, ISSN 0008-8749 Journal Code: 1246405

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Interleukin-2 (IL-2) is a lymphokine synthesized by T cells following activation. Resting T cells do not express IL-2 receptors, but receptors are rapidly expressed on T cells following interaction of the antigen-specific T-cell-receptor complex with appropriately processed and presented antigens. Anti-Tac, a monoclonal antibody that recognizes the IL-2 receptor, has been used to purify the receptor. The receptor is a 55-kDa glycoprotein comprised of 251 amino acids including a single

19-amino transmembrane domain and a short intracytoplasmic domain composed of 13 amino acids at the carboxy terminus. Normal resting T cells and most leukemic T-cell populations examined did not express IL-2 receptors; however, the leukemic cells of all patients with human T-cell lymphotropic virus (HTLV-I)-associated adult T-cell leukemia (ATL) expressed the Tac antigen. In HTLV-I-infected cells, the 42-kDa long open reading frame (tat) protein encoded in part by the tat region of HTLV-I may act as a transacting activator that induces **transcription** of the IL-2-receptor gene, thus providing an explanation for the constant association of HTLV-I infection of lymphoid cells and IL-2-receptor expression. The constant display of large numbers of IL-2 receptors which may be aberrant in the ATL cells may play a role in the uncontrolled growth of these leukemic T cells. Patients with the Tac-positive ATL are being **treated** with both unmodified and toxin-conjugated forms of anti-Tac monoclonal antibody directed toward this growth **factor** receptor.

18/3,AB/115 (Item 115 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

05095346 86168470 PMID: 3958045

Increase in urokinase plasminogen activator mRNA synthesis in human carcinoma cells is a primary effect of the potent tumor promoter, phorbol myristate acetate.

Stoppelli M P; Verde P; Grimaldi G; Locatelli E K; Blasi F
Journal of cell biology (UNITED STATES) Apr 1986, 102 (4)
p1235-41, ISSN 0021-9525 Journal Code: 0375356
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The effect of tumor promoters and growth **factors** on the synthesis of urokinase and urokinase mRNA in human carcinoma cells has been investigated. In urokinase-producing human carcinoma cells (A1251), a 20-40-fold increase in urokinase mRNA level is obtained after **treatment** with 10 nM phorbol myristate acetate (PMA), a smaller effect (two- to fourfold) with 2 ng/ml platelet-derived growth **factor** (PDGF) and no effect with epidermal growth **factor** (EGF) (up to 50 nM). After **treatment** with PMA, urokinase mRNA level increases already at 30 min peaking 2-4 h thereafter. Cell line A431, which has an abnormally high number of EGF receptors, shows the same response to PMA, but also responds to EGF (two- to fourfold increase in mRNA). The kinetics are similar to those of A1251. Nuclear **transcription** experiments show that the PMA-induced increase in urokinase mRNA is due to increased synthesis. The protein synthesis inhibitor, cycloheximide (10 micrograms/ml), also increases the level of urokinase mRNA. When both cycloheximide and PMA are used, super-induction is observed. This result may indicate that a short-lived protein negatively regulates the level of urokinase. The different efficiency of the effectors (PMA and PDGF better than EGF) and their kinetics, as well as the effect of cycloheximide on urokinase mRNA synthesis, (a) are reminiscent of the effect of PDGF and PMA on competence phase genes (Kelly, K., B.H. Cochran, C.D. Stiles, and P. Leder, 1983, Cell, 35: 603-610), (b) demonstrate that the synthesis of urokinase is part of the early cellular response to these **factors**, and (c) provide a preliminary insight in the overproduction of urokinase by primary **malignant** tumors and transformed cells in culture.

18/3,AB/116 (Item 116 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

04455235 84142301 PMID: 6322013

Tumour induction in the rodent *Mastomys natalensis* by activation of endogenous papilloma virus genomes.

Amtmann E; Volm M; Wayss K
Nature (ENGLAND) Mar 15-21 1984, 308 (5956) p291-2, ISSN
0028-0836 Journal Code: 0410462
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Specific DNA sequences from human papillomavirus have recently been detected in carcinomas from epidermodysplasia verruciformis patients, and in vulvar and cervical carcinomas but the role of papilloma viruses in the aetiology of these tumours is unclear. Indeed, little is known about the mechanisms that convert benign papillomas into **malignant** tumours and it is not even possible in tumour induction. Here, we describe an animal system that permits an analysis of the interaction of papilloma virus genomes with carcinogenic agents at the molecular level. In our colony of *Mastomys natalensis* (a close relative of the rat family), we have found extrachromosomal papilloma virus genomes persisting in a variety of tissues such as skin, muscle, liver and colon. With the ageing of the animals, the average copy number of viral DNA in skin cells increases and virus-producing tumours begin to appear in *Mastomys* at about 1 year old. This process is drastically enhanced by chronic **treatment** with a tumour promoter and **transcription** of the viral genomes has been found to be correlated with tumour formation.

18/3,AB/117 (Item 117 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

04100147 83076866 PMID: 6293699
Hormonal and metabolic regulation of human chondrosarcoma in vitro.
McCumbee W D; Harrelson J M; Lebovitz H E
Cancer research (UNITED STATES) Feb 1983, 43 (2) p513-6,
ISSN 0008-5472 Journal Code: 2984705R
Contract/Grant No.: AM01324; AM; NIADDK
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Prostaglandin A1 has a profound inhibitory effect on uridine incorporation into RNA of normal cartilage whereas N6-monobutyryladenosine 3',5'-cyclic monophosphate is either stimulatory or without an effect. Sera from intact and growth hormone-**treated** hypophysectomized rats stimulate RNA synthesis but serum from untreated hypophysectomized rats does not. The present study investigated the in vitro regulation of [3H]uridine incorporation into RNA of six human chondrosarcomas to determine if **malignant** human chondrocytes are under similar metabolic and hormonal regulation. Prostaglandin A1 (25 micrograms/ml) markedly inhibited uridine incorporation in all six tumors (56 to 80%). N6-Monobutyryladenosine 3',5'-cyclic monophosphate (1 mM) inhibited uridine incorporation in five tumors (20 to 50%). Uridine incorporation was stimulated by growth hormone-dependent serum **factors** in one tumor and by growth hormone-independent serum **factors** in two tumors. Two tumors were more responsive to serum from growth hormone-**treated** hypophysectomized rats than to serum from intact rats, and one tumor was unresponsive to serum stimulation. The data indicate that: (a) prostaglandin A1 is a very potent inhibitor of RNA synthesis in human chondrosarcomas; (b) N6-monobutyryladenosine 3',5'-cyclic monophosphate affects human chondrosarcomas differently than it does normal cartilage; and (c) responses of human chondrosarcomas to serum growth **factors** vary among individual tumors.

18/3,AB/118 (Item 118 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

03471568 81013947 PMID: 6251475

Passive immunotherapy prevents expression of endogenous Moloney virus and amplification of proviral DNA in BALB/Mo mice.

Nobis P; Jaenisch R

Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Jun 1980, 77 (6) p3677-81, ISSN

0027-8424 Journal Code: 7505876

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

BALB/Mo mice carrying the Moloney murine leukemia virus (M-MuLV) as an endogenous virus become viremic soon after birth and develop leukemia at a later age. M-MuLV-specific gene expression and an increase of virus-specific DNA copies in lymphatic target organs are characteristics of the preleukemic phase. Passive immunotherapy of new born BALB/Mo mice with anti-gp70 glycoprotein or anti-M-MuLV serum prevented viremia and delayed significantly the subsequent development of leukemia. Molecular hybridization experiments showed that both virus-specific genome **transcription** and virus-specific DNA amplification could be completely suppressed by antiserum **treatment**. Thus virus-specific RNA concentrations in target organs of immunized BALB/Mo mice of 6 months or older were as low as in normal BALB/c mice. This is an age at which untreated BALB/Mo mice have already developed **malignant** lymphoma. Our experiments demonstrate that **treatment** with antiserum interferes with the early events of virus expression and thus prevents the subsequent steps leading to leukemia.

18/3,AB/119 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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11227034 BIOSIS NO.: 199800008366

Role of DNA mismatch repair in the cytotoxicity of ionizing radiation.

AUTHOR: Fritzell James A; Narayanan Latha; Baker Sean M; Bronner C Eric;
Andrew Susan E; Prolla Tomas A; Bradley Allan; Jirik Frank R; Liskay R
Michael; Glazer Peter M(a)

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208040, New Haven, CT 06520-8040**USA

JOURNAL: Cancer Research 57 (22):p5143-5147 Nov. 15, 1997

ISSN: 0008-5472

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: The DNA mismatch repair (MMR) system in mammalian cells not only serves to correct base mispairs and other replication errors, but it also influences the cellular response to certain forms of DNA damage. Cells that are deficient in MMR are relatively resistant to alkylation damage because, in wild-type cells, the MMR system is thought to promote toxicity via futile repair of alkylated mispairs. Conversely, MMR-deficient cells are sensitive to UV light, possibly due to the requirement for MMR **factors** in **transcription-coupled** repair of active genes. MMR deficiency has been associated with familial and sporadic carcinomas of the colon and other sites, and so, we sought to determine the influence of MMR status on cellular response to ionizing radiation, an agent commonly used for cancer therapy. Fibroblast cell lines were established from transgenic mice carrying targeted disruptions of one of three MMR genes in mammalian cells: Pms2, Mlh1, or Msh2. In comparison to wild-type cell lines from related mice, the Pms2-, Mlh1-, or Msh2-nullizygous cell lines were found to exhibit higher levels of clonogenic survival following exposure to ionizing radiation. Because

ionizing radiation generates a variety of lesions in DNA, the differences in survival may reflect a role for MMR in processing a subset of these lesions, such as damaged bases. These results both identify a new class of DNA-damaging agents whose effects are modulated by the MMR system and may help to elucidate pathways of radiation response in cancer cells.

1997

18/3,AB/120 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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11226439 BIOSIS NO.: 199800007771

Induction of activating **transcription factor** 1 by nickel and its role as a negative regulator of thrombospondin I gene expression.

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JOURNAL: Cancer Research 57 (22):p5060-5066 Nov. 15, 1997

ISSN: 0008-5472

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Thrombospondin I (TSP I) is an extracellular matrix glycoprotein that influences cell adhesion, motility, and growth. On the basis of its effects on endothelial cell proliferation, TSP I has attracted interest as a potential regulator of solid tumor growth through modulation of tumor blood supply. The regulation of TSP I expression is of critical importance for designing new approaches in tumor therapy. Recently, we have shown that TSP I expression is lost in nickel-transformed cells. In this paper, we identified an activating **transcription factor** (ATF)/cAMP-responsive element-binding protein binding site as a negative regulatory site in the 5'-flanking sequence of mouse TSP I promoter. We identified ATF-1 as a major component of the ATF/cAMP-responsive element-binding protein binding complex. This M|r 35,000 nuclear ATF-1 protein was shown to be present in higher amounts in nickel-transformed 3T3 cells that do not express TSP I. Acute **treatment** of 3T3 cells with NiCl₂ resulted in the induction of this **transcription factor**, and this induction was correlated temporally with the suppression of TSP I expression in the same cells. These results show that nickel exposure causes accumulation of the ATF-1 **transcription factor**, which is responsible for the down-regulation of **transcription** of TSP I, and possibly other tumor suppressor genes during nickel-induced cellular transformation.

1997

18/3,AB/121 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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10884145 BIOSIS NO.: 199799505290

Induction of **transcription factor** NF-kappa-B by tumor necrosis **factor** alpha and interferon beta in human glioma cell lines.

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JOURNAL: Environmental Medicine (Nagoya) 40 (1):p25-29 1996

ISSN: 0287-0517

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Tumor necrosis **factor** alpha (TNF-alpha) and interferon beta (INF-beta) are used to **treat** cancers such as human **malignant** gliomas. However, susceptibility to these cytokines varies among glioma cells, limiting the validity of **treatment**. To clarify the underlying mechanism for the different susceptibility to TNF-alpha and INF-beta, NF-kappa-B activities induced by these cytokines were analyzed in two glioma cell lines: SK-MG1 sensitive to TNF-alpha and IFN-beta, and U251-MG resistant to the cytokines. Under a non-stimulated state, NF-kappa-B activity determined by electrophoretic mobility shift assay (EMSA) was detected in SK-MG1 cells, but was absent in U251-MG cells. **Treatment** with TNF-alpha and/or INF-beta induced NF-kappa-B activity in both cell lines, especially as a single complex in SK-MG1 cells and as two complexes in U251-MG cells. Analysis by antisera specific to NF-kappa-B components in EMSA indicated that the single complex in SK-MG1 cells includes a p50 homodimer, while the complexes in U251-MG cells show a p50 homodimer as well as a p50-p65 heterodimer. Since a p50-p65 heterodimer activates NF-kappa-B responsive genes, it is possible that the induction of NF-kappa-B responsive genes by these cytokines may serve to protect the cells from the cytotoxic effects of cytokines.

1996

18/3,AB/122 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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10651826 BIOSIS NO.: 199699272971

Regulation of the redox-sensitive **transcription factors** NF-kB and AP-1 by ultraviolet light-B in human melanocytes.

BOOK TITLE: International Congress Series; Melanogenesis and **malignant** melanoma: Biochemistry, cell biology, molecular biology, pathophysiology, diagnosis and **treatment**

AUTHOR: Meyskens Frank L Jr(a); Buckmeier Julie; Tohidian Nilou

BOOK AUTHOR/EDITOR: Hori Y; Hearing V J; Nakayama J: Eds

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JOURNAL: International Congress Series (1096):p25-34 **1996**

BOOK PUBLISHER: Elsevier Science Publishers B.V., PO Box 211, Sara Burgerhartstraat 25, 1000 AE Amsterdam, Netherlands
Elsevier Science Publishing Co., Inc., P.O. Box 882, Madison Square Station, New York, New York 10159-2101, USA

CONFERENCE/MEETING: International Symposium on Melanogenesis and Malignant Melanoma Fukuoka, Japan December 4-6, 1995

ISSN: 0531-5131 **ISBN:** 0-444-82209-7

RECORD TYPE: Citation

LANGUAGE: English

1996

18/3,AB/123 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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10566647 BIOSIS NO.: 199699187792

Curable and noncurable malignancies: Lessons from paediatric cancer.

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JOURNAL: Medical Oncology (London) 13 (1):p15-21 **1996**

ISSN: 1357-0560
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The tremendous progress achieved in understanding the molecular basis of cancer, was unfortunately not followed by a mutual improvement in the morbidity and mortality of adult cancer. In contrast, the success rate achieved in paediatric oncology has increased significantly during the past 30 years, and more than two-thirds of the children with cancer can now be cured. p53 has been shown to have a central role on apoptosis in various cells. As apoptosis is a final common pathway for much of our anti cancer therapy, resistance to apoptosis due to a normal activity of p53 is an important mechanism of tumor resistance and **treatment** failure. Contrary to the findings in most adult tumors, where about 50% of the tumors lack p53 activity, the rate of p53 mutations in childhood cancer is surprisingly low. This may be the key to the much better prognosis of children with cancer. In most adult tumors, multiple genetic events, between five and seven, are usually involved. The oncogenes involved in such tumors usually represent those located upstream of the nuclear **transcription factors**. In most paediatric tumors, in contrast, the initiating event is the activation of nuclear **transcription factors** secondary to chromosomal translocations. It can be speculated that multiple events activating various components of the signal transduction machinery are needed for tumorigenesis, and hence the evolution and progression of such tumors is slow. Moreover, if the **malignant** cell has to accumulate multiple mutations, the chances of crippling the apoptotic mechanism are higher. Genomic instability evidenced by microsatellite variation has been found in colon, pancreas, breast, liver and ovarian adult tumors, and not in paediatric tumors. As multiple somatic mutations are needed for the initiation and progression of the common adult malignancies, inherent genomic instability can dispose to accumulation of multiple mutations. All these molecular interactions are discussed with relevance to the difference between non-curable, mostly adult tumors, and curable, mostly paediatric tumors.

1996

18/3,AB/124 (Item 6 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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10386997 BIOSIS NO.: 199699008142

A comparative study of cytokine gene transcripts in normal and **malignant** breast tissue and primary cell cultures derived from the same tissues samples.

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JOURNAL: International Journal of Cancer 66 (4):p551-556 1996

ISSN: 0020-7136

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Using a differential centrifugation method followed by culture in selective medium, we have successfully isolated and maintained individual epithelial and stromal cells from normal (n = 10) and **malignant** (n = 6) human breast tissue and characterized their phenotype by immunocytochemistry. Further, we have studied expression of the cytokine genes IL-1-beta, IL-6, IL-8 and TNF-beta in each cell fraction by RT-PCR and have compared these results with cytokine gene expression in tissue extracts from which primary cultures were derived. In breast tumours, there was near complete absence of IL-1-beta in both whole tissue and

cell fractions, and in normals it was present in only 3/10 tissue preparations, with increased expression in stromal (6/10) and epithelial (5/10) cell samples. IL-6 was constitutively expressed in all tumour-derived breast tissue samples but downregulated in tumour cell cultures, with the opposite result in normal breast. Near identical levels of IL-8 expression were found throughout each preparation, irrespective of tissue origin. TNF-beta was expressed in all normal tissue samples, in 9/10 epithelial preparations but in only 6/10 stromal preparations. In tumours, TNF-beta was associated predominantly with whole tissue or stromal samples, with reduced expression in epithelial preparations. Our data confirm that primary cultures of normal and **malignant** human breast tissue can be successfully separated into epithelial and mesenchymal cell populations and their phenotype can be maintained in culture for up to 30 days. However, this cellular separation does alter the cytokine profiles; therefore, experimental findings with isolated cells should be **treated** with a caveat.

1996

18/3,AB/125 (Item 7 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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10339585 BIOSIS NO.: 199698794503

Induction of **transcription factor** NF-kappa-B by tumor necrosis **factor** alpha (TNF-alpha) in human glioma cell lines: Correlation with TNF-alpha susceptibility.

AUTHOR: Nagaya Takashi(a); Sato Motomi; Yoshida Jun; Fujieda Miyuki; Seo Hisao

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JOURNAL: Environmental Medicine (Nagoya) 39 (1):p13-16 1995

ISSN: 0287-0517

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Tumor necrosis **factor** alpha (TNF-alpha) is applied in the **treatment** of such cancers as human **malignant** gliomas, even though its efficacy is limited against certain types of gliomas depending on the susceptibility of the cells. To clarify the underlying mechanism involved in the different susceptibility to TNF-alpha, NF-kappa-B activities induced by TNF-alpha were analyzed in two glioma cell lines using gel mobility shift assays. While NF-kappa-B activity was detected in the absence of TNF-alpha among TNF-alpha-sensitive SK-MG1 cells, none could be found among TNF-alpha-resistant U251-MG cells. TNF-alpha induced NF-kappa-B activity in both cell lines, especially with an additional complex in U251-MG cells. These results suggest that the basal expression of NF-kappa-B in SK-MG1 cells or the different NF-kappa-B complex induced by TNF-alpha in U251-MG cells might be related to the varying degrees of susceptibility of tumor cells to TNF-alpha.

1995

18/3,AB/126 (Item 8 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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10291461 BIOSIS NO.: 199698746379

Keratinocyte growth **factor** enhances urokinase-type plasminogen activator activity in HPV16 DNA-immortalized human uterine exocervical epithelial cells.

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JOURNAL: European Journal of Cell Biology 69 (2):p128-134 1996
ISSN: 0171-9335
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We recently demonstrated that keratinocyte growth factor (KGF), a fibroblast-derived growth factor, induced anchorage-independent growth of HPV16 DNA-immortalized human uterine exocervical epithelial cells (HCE16/3 cell line) in soft agarose assay. We have now studied whether KGF might also regulate the plasminogen activator (PA) system, another transformation parameter related to cell invasiveness. HCE16/3 cells were found to produce urokinase-type PA (uPA) and small amounts of tissue-type PA (tPA) as determined by immunocapture assay. Secretion of both uPA and tPA was increased when HCE16/3 cells were exposed to KGF for 4 h and continued to increase during the next 24 h. The early increase following KGF treatment was presumably mediated by binding of KGF to its receptors. Enhanced secretion of uPA in HCE16/3 cells by KGF was also seen by zymographic analysis and immunofluorescence. According to Northern blotting KGF upregulated the transcription of uPA gene in HCE16/3 cells; the upregulation occurred only after 12 h of KGF treatment, suggesting that stimulation of uPA production by KGF is a biphasic event. As proteolysis is a prerequisite for tumor cell invasion, taken together with our previous results, the present findings suggest that KGF may play an important role in the transition of immortalized uterine cervical epithelial cells from in situ to invasive carcinoma.

1996

18/3,AB/127 (Item 9 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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09688070 BIOSIS NO.: 199598142988
Actions of vitamin D-3 analogs on human prostate cancer cell lines:
Comparison with 1,25-dihydroxyvitamin D-3.
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JOURNAL: Endocrinology 136 (1):p20-26 1995
ISSN: 0013-7227
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Data from epidemiological studies has suggested that vitamin D deficiency may promote prostate cancer, although the mechanism is not understood. We have previously demonstrated the presence of vitamin D receptors (VDR) in three human prostate carcinoma cell lines (LNCaP, PC-3, and DU-145) as well as in primary cultures of stromal and epithelial cells derived from normal and malignant prostate tissues. We have also shown that 1,25-dihydroxyvitamin D-3 (1,25-(OH)-2D-3) can elicit an antiproliferative action in these cells. In the present study we compared the biological actions of 1,25-(OH)-2D-3 to those of a series of natural vitamin D-3 metabolites and several synthetic analogs of vitamin D-3 known to exhibit less hypercalcemic activity in vivo. In ligand binding competition experiments, we demonstrated the following order of potency in displacing (3H)1,25(OH)-2D-3 from VDR: EB-1089 gt 1,25-(OH)-2D-3 gt MC-903 gt

1,24,25-(OH)-3D-3 gt 22-oxacalcitriol (OCT) gt
 1-alpha,25-dihydroxy-16-enecholecalciferol (Ro24-2637) gt
 25-hydroxyvitamin D-3, with EB-1089 being -2-fold more potent than the native hormone. No competitive activity was found for 25-hydroxy-16,23-diene-cholecalciferol. When compared for ability to inhibit proliferation of LNCaP cells, MC-903, EB-1089, OCT, and Ro24-2637 exhibited 4-, 3-, 3-, and 2-fold greater inhibitory activity than 1,25-(OH)-2D-3. Interestingly, although OCT and Ro24-2637 exhibit, respectively, 10 and 14 times lower affinity for VDR than 1,25-(OH)-2D-3, both compounds inhibited the proliferation of LNCaP cells with a potency greater than that of the native hormone. The relative potency of vitamin D-3 metabolites and analogs to inhibit cell proliferation correlated well with the ability of these compounds to stimulate prostate-specific antigen secretion by LNCaP cells as well as with their potency to induce the 25-hydroxyvitamin D-3-24-hydroxylase messenger RNA transcript in PC-3 cells. In conclusion, these results demonstrate that synthetic analogs of vitamin D-3, known to exhibit reduced calcemic activity, can elicit antiproliferative effects and other biological actions in LNCaP and PC-3 cell lines. It is noteworthy that although binding to VDR is critical for 1,25-(OH)-2D-3 action, the analog data indicate that additional **factors** significantly contribute to the magnitude of the biological response. Finally, the strong antiproliferative effects of several synthetic analogs known to exhibit less calcemic activity than 1,25(OH)₂D₃ suggest that these compounds potentially may be useful as an additional therapeutic option for the **treatment** of prostate cancer.

1995

18/3,AB/128 (Item 10 from file: 5)
 DIALOG(R) File 5: Biosis Previews(R)
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09639982 BIOSIS NO.: 199598094900

Interferon-stimulated response element and NF-kappa-B sites cooperate to regulate double-stranded RNA-induced **transcription** of the IP-10 gene.

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JOURNAL: Journal of Interferon Research 14 (6):p357-363 1994

ISSN: 0197-8357

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: To understand the mechanisms involved in dsRNA-induced gene expression, we analyzed the poly(I/C)-induced **transcription** of the IFN-inducible chemokine gene IP-10 using the GRE cell line in which type I IFN genes have been deleted. Accumulation of IP-10 mRNA in GRE cells was more strongly stimulated by **treatment** with dsRNA than by IFN-alpha or IFN-gamma and was independent of protein synthesis. This same pattern of response was produced when GRE cells were transiently transfected with a plasmid containing 243 bases of sequence from the promoter of the murine IP-10 gene linked to the chloramphenicol acetyltransferase reporter gene. Deletion- and site-specific mutagenesis of the 243 base pair fragment indicated that an ISRE located between residues -204 and -228 was a primary target site for the action of dsRNA on this promoter. This was confirmed by results showing that two copies of this ISRE tandemly arrayed in front of the thymidine kinase promoter were able to mediate reporter gene **transcription** in dsRNA-stimulated cells. At least one of the two NF-kappa-B binding sites present in the 243 base pair IP-10 promoter is also necessary for

response to dsRNA; mutation of both sites eliminates promoter activity. Thus the ISRE and one NF-kappa-B site cooperate to produce transcriptional response to dsRNA.

1994

18/3,AB/129 (Item 11 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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08335439 BIOSIS NO.: 000094086687
ONCOGENES ONCOPROTEINS AND TUMOR SUPPRESSOR GENES IMPORTANCE FOR
CARCINOGENESIS
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JOURNAL: DMW (DTSCH MED WOCHENSCHR) 116 (41). 1991. 1563-1568. 1991
FULL JOURNAL NAME: DMW (Deutsche Medizinische Wochenschrift)
CODEN: DDMWD
RECORD TYPE: Abstract
LANGUAGE: GERMAN

ABSTRACT: Studies on the transmissibility of tumors by viruses led to the discovery of oncogenes. Gene fragments (v-onc) which caused transformation were detected in viral genomes. Homologous genes (c-onc) were found in cellular genomes. Proliferation and cell metabolism are controlled by these genes (proto-oncogenes). They code nuclear **factors** involved in **transcription** as well as protein kinase, hormone and growth **factor** receptors, growth **factors** and proteins involved in signal transmission. Dysfunction of gene expression (point mutation, amplification, insertion and translocation) turns proto-oncogenes into oncogenes. When the regulation of cell growth is disturbed, immortalization and transformation occur. If at the same time tumor suppressor genes (anti-oncogenes) are lost by allele deletion, the degeneration is **malignant**. The stepwise accumulation of genetic changes was discovered in colorectal adenoma-carcinoma sequence. The knowledge of molecular biological mechanisms of carcinogenesis will improve the preventive and follow-up **treatment** of cancer patients. Production of antibodies which bind oncogenes is a promising starting point for therapy.

1991

18/3,AB/130 (Item 12 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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06586193 BIOSIS NO.: 000087028355
TRANSFORMING GROWTH **FACTOR** BETA-1 AND CYCLIC AMP INHIBIT
TRANSCRIPTION OF EPIDERMAL GROWTH **FACTOR** AND ONCOGENE-INDUCED
TRANSIN RNA
AUTHOR: KERR L D; OLASHAW N E; MATRISIAN L M
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TENN. 37232.
JOURNAL: J BIOL CHEM 263 (32). 1988. 16999-17005. 1988
FULL JOURNAL NAME: Journal of Biological Chemistry
CODEN: JBCHA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Transin mRNA encodes a secreted metalloprotease which is transcriptionally induced in Rat-1 cells by epidermal growth **factor**

(EGF) and a number of oncogenes. A role for transin in tumor progression is suggested by its overexpression in **malignant** and metastatic tumors compared to their benign counterparts. In an effort to elucidate mechanisms by which elevated transin expression may be inhibited, it has been determined that both transforming growth **factor** type .beta.1 (TGF.beta.1) and increased levels of intracellular cyclic 5'- adenosine monophosphate (cAMP) inhibit EGF and oncogene induction of transin mRNA. The inhibition of transin mRNA occurred at the level of **transcription** as demonstrated by nuclear run-on assays. EGF binding studies in Rat-1 cells showed no significant effect of cAMP or TGF.beta.1 on EGF receptor number or affinity. We have also examined the effects of cAMP and TGF.beta.1 on oncogene-induced transin using Rat-1 cells transformed by temperature-sensitive mutants of v-src and K-ras oncogenes. Both inhibitors prevented the induction of transin RNA as well as decreased the levels of transin once elevated at the permissive temperature. Despite the similarities in the actions of TGF.beta.1 and cAMP on transin gene expression, TGF.beta.1 **treatment** did not significantly elevate intracellular cAMP levels, thus making it unlikely that cAMP is a second messenger system for TGF.beta.1 action. These studies suggest that the inhibitory effects of cAMP and TGF.beta.1 occur by distinct pathways at the level of gene regulation.

1988

18/3,AB/131 (Item 13 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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06237431 BIOSIS NO.: 000086071613

CHARACTERIZATION OF HUMAN RECOMBINANT TUMOR NECROSIS **FACTOR**-ALPHA
ANTIPROLIFERATIVE EFFECTS ON HUMAN CELLS IN CULTURE

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77030.

JOURNAL: LYMPHOKINE RES 7 (2). 1988. 107-118. 1988

FULL JOURNAL NAME: Lymphokine Research

CODEN: LYRED

RECORD TYPE: Abstract

LANGUAGE: ENGLISH

ABSTRACT: TNF is a macrophage-derived polypeptide known to cause hemorrhagic necrosis of transplanted tumors and is cytotoxic or cytostatic to a variety of **malignant** human cells in vitro. However, little is known about its mechanism of antiproliferative activity. Human cervical carcinoma (ME-180) cells in log-phase were **treated** with various doses of rTNF (from Genentech, Inc., Sp. Act. 5.7 .times. 10⁷ U/mg) for 72 hours. Fifty percent inhibition of cell growth was obtained at a dose of 1000 U/ml (ID50). An identical ID50 was obtained for confluent cells. Maximal antiproliferative effects were observed only with 72 hours continuous exposure to rTNF. Exposure of cells to an ID50 dose for 12 hours or less resulted in no antiproliferative effect; similar to results obtained with murine TNF. The incorporation of [3H] uridine and [3H] thymidine was reduced by 25 and 40% respectively by 24 hours after TNF **treatment**. [3H] uridine incorporation rebounded to 170% of controls level 72 hours after TNF addition. There was a transient 40% decrease in [3H] leucine incorporation at 48 hours which recovered to control value by 72 hours. These studies show that TNF can suppress both DNA and RNA synthesis while protein synthesis is only transiently affected. Considering its short plasma half-life (.apprx. 20 min) in vivo, these studies also suggest that for optimal antiproliferative effect rTNF should be administered in a fashion which provides sustained drug levels.

1988

18/3,AB/132 (Item 14 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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06064045 BIOSIS NO.: 000085027194
BIOLOGICAL PROPERTIES OF SUPPRESSIVE E-RECEPTOR **FACTOR** 1 LYMPHOKINE
FUNCTION

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JOURNAL: EUR J IMMUNOL 17 (10). 1987. 1403-1410. 1987
FULL JOURNAL NAME: European Journal of Immunology
CODEN: EJIMA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: A potent immunosuppressive **factor** isolated from **malignant** ascites fluids showed serological cross-reactivity with the E-receptor of human peripheral blood T lymphocytes. Thus, this **factor** was named suppressive E-receptor (SER) **factor**. In this study, we examined the effect of this immunosuppressor, SER, on lymphokine functions of human mononuclear cells participating in polyclonal T cell activation. SER is active at nanomolar concentrations in vitro and the inhibitory effect of SER was most pronounced when added at the initiation of stimulation with phytohemagglutinin or anti-T3 antibody. Concomitant with the inhibition on PHA-induced DNA synthesis, lymphocytes that were **treated** with SER failed to progress beyond G1 phase of cell cycle. These growth-arrested cells did expire after 7 days of culture in vitro. This anti-proliferative effect of SER was more easily demonstrated with normal lymphoid cells in culture than transformed cells or fibroblast cells. SER effectively interfered with the lympho-proliferative properties of interleukin 2 (IL 2) on human peripheral blood mononuclear cells and an IL 2-dependent murine cytotoxic T cell line. However, excess quantities of exogenous IL 2, especially when added in conjunction with IL 1, were able to partially overcome the ability of SER to inhibit T cell proliferation. In contrast to the inhibition on DNA synthesis of human lymphoblasts, expression of IL 2 receptor was only minimally inhibited by SER during the first 24 h of culture (24% inhibition at 12 h and 34% inhibition at 24 h) but it was followed by full expression of IL 2 receptor by 48 h. Thus, SER merely reduced the rate of expression of IL 2 receptor and was not able to inhibit the **transcription** of new message from activated T lymphocytes. Taken together, these studies indicate that SER acts as a noncytolytic anti-proliferative **factor** on immune responses that are mediated by T cells. SER appears to act on a relatively late event during T cell activation, perhaps on some portion of the DNA replication pathway.

1987

18/3,AB/133 (Item 15 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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05719784 BIOSIS NO.: 000084068190
RAPID AND SELECTIVE ALTERATIONS IN THE EXPRESSION OF CELLULAR GENES
ACCOMPANY CONDITIONAL **TRANSCRIPTION** OF HA-V-RAS IN NIH 3T3 CELLS
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N.C. 27706.
JOURNAL: MOL CELL BIOL 7 (7). 1987. 2512-2520. 1987
FULL JOURNAL NAME: Molecular and Cellular Biology
CODEN: MCEBD
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Hormone **treatment** of NIH 3T3 cells that contain recombinant fusions between the mouse mammary virus long terminal repeat and the v-ras gene of Harvey murine sarcoma virus results in conditional expression of the ras p21 gene product. Levels of ras mRNA and p21 are maximal after 2 to 4 h of hormone **treatment**. Analysis of cellular RNA by Northern blotting and nuclease S1 protection assays indicates that the expression of two cellular RNA species increases with kinetics similar to v-ras: v-sis-related RNA and retrovirus-related VL30 RNA. Run-on **transcription** in isolated nuclei shows that the increase in v-sis-related RNA is not dependent on **transcription** and therefore must arise by a post-transcriptional mechanism. The increase in VL30 expression is a transcriptional effect. Hormone **treatment** of normal NIH 3T3 cells has no effect on the expression of these DNA sequences. These results suggest that v-ras stimulation of autocrine **factors** may play a role in transformation of cells by this gene and also suggest a reverse genetic strategy to determine the nucleic acid sequences and cellular **factors** involved in the regulation of gene expression that is observed.

1987

18/3,AB/134 (Item 16 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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05184791 BIOSIS NO.: 000082025412
INCREASE OF UROKINASE PLASMINOGEN ACTIVATOR MESSENGER RNA SYNTHESIS IN HUMAN CARCINOMA CELLS IS A PRIMARY EFFECT OF THE POTENT TUMOR PROMOTER PHORBOL MYRISTATE ACETATE
AUTHOR: STOPPELLI M P; VERDE P; GRIMALDI G; LOCATELLI E K; BLASI F
AUTHOR ADDRESS: INTERNATIONAL INST. GENETICS AND BIOPHYSICS, CNR, VIA MARCONI, 10, 80125 NAPLES, ITALY.
JOURNAL: J CELL BIOL 102 (4). 1986. 1235-1241. 1986
FULL JOURNAL NAME: Journal of Cell Biology
CODEN: JCLBA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The effect of tumor promoters and growth **factors** on the synthesis of urokinase and urokinase mRNA in human carcinoma cells has been investigated. In urokinase-producing human carcinoma cells (A1251), a 20-40-fold increase in urokinase mRNA level is obtained after **treatment** with 10 nM phorbol myristate acetate (PMA), a smaller effect (two- to fourfold) with 2 ng/ml platelet-derived growth **factor** (PDGF) and no effect with epidermal growth **factor** (EGF) (up to 50 nM). After **treatment** with PMA, urokinase mRNA level increases already at 30 min peaking 2-4 h thereafter. Cell line A431, which has an abnormally high number of EGF receptors, shows the same response to PMA, but also responds to EGF (two- to fourfold increase in mRNA). The kinetics are similar to those of A1251. Nuclear **transcription** experiments show that the PMA-induced increase in urokinase mRNA is due to increased synthesis. The protein synthesis inhibitor, cycloheximide (10 .mu.g/ml), also increases the level of urokinase mRNA. When both cycloheximide and PMA are used, superinduction is observed. This result may indicate that a short-lived protein negatively regulates the level of urokinase. The different efficiency of

the effectors (PMA and PDGF better than EGF) and their kinetics, as well as the effect of cycloheximide on urokinase mRNA synthesis, (a) are reminiscent of the effect of PDGF and PMA on competence phase genes (Kelly, K., B. H. Cochran, C.D. Stiles, and P. Leder, 1983, Cell, 35:603-610), (b) demonstrate that the synthesis of urokinase is part of the early cellular response to these **factors**, and (c) provide a preliminary insight in the overproduction of urokinase by primary **malignant** tumors and transformed cells in culture.

1986

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removal, customized scheduling. See HELP ALERT.

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7/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10042256 99024018 PMID: 9804857
Angiotensin II-induced tyrosine phosphorylation of signal transducers and
activators of **transcription** 1 is regulated by Janus-activated kinase
2 and Fyn kinases and mitogen-activated protein kinase phosphatase 1.
Venema R C; Venema V J; Eaton D C; Marrero M B
Vascular Biology Center, Medical College of Georgia, Augusta, Georgia
30912, USA.
Journal of biological chemistry (UNITED STATES) Nov 13 1998, 273
(46) p30795-800, ISSN 0021-9258 Journal Code: 2985121R
Contract/Grant No.: HL57201; HL; NHLBI; HL58129; HL; NHLBI; P01-DK50268;

DK; NIDDK

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Angiotensin II (Ang II) AT1 receptors on vascular smooth muscle cells (VSMCs) are coupled to the Janus-activated kinase (JAK)/signal transducers and activators of **transcription** (STAT) pathway. We have shown previously that Ang II stimulation of VSMCs results in the tyrosine phosphorylation of JAK2 and STAT1 and the translocation of STAT1 to the nucleus. In the present study, we demonstrate using specific enzyme inhibitors and antisense **oligonucleotides** that both JAK2 and p59 Fyn tyrosine kinases are required for the Ang II-induced tyrosine phosphorylation and nuclear translocation of STAT1 in VSMCs. Neither tyrosine kinase, however, appears to function upstream from the other in a phosphorylation cascade. Rather, p59 Fyn functions as an Ang II-activated docking protein for both JAK2 and STAT1, a docking interaction that may facilitate JAK2-mediated STAT1 tyrosine phosphorylation. In this study, we have also identified the nuclear dual-specificity phosphatase mitogen-activated protein kinase phosphatase 1 as the enzyme responsible for STAT1 tyrosine dephosphorylation in VSMCs.

7/3,AB/2 (Item 2 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

10007666 98443229 PMID: 9769331

Requirement of Stat3 but not Stat1 activation for epidermal growth factor receptor-mediated cell growth In vitro.

Grandis J R; Drenning S D; Chakraborty A; Zhou M Y; Zeng Q; Pitt A S; Tweardy D J

Department of Otolaryngology, University of Pittsburgh School of Medicine and the University of Pittsburgh Cancer Institute, Pittsburgh, PA 15213, USA. jgrandis@vms.cis.pitt.edu

Journal of clinical investigation (UNITED STATES) Oct 1 1998,
102 (7) p1385-92, ISSN 0021-9738 Journal Code: 7802877

Contract/Grant No.: CA 72261; CA; NCI; CA01760; CA; NCI; CA72526; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Stimulation of epidermal growth factor receptor (EGFR) by ligand(s) leads to activation of signaling molecules including Stat1 and Stat3, two members of the signal transducers and activators of **transcription** (STAT) protein family. Activation of Stat1 and Stat3 was constitutive in transformed squamous epithelial cells, which produce elevated levels of TGF-alpha, and was enhanced by the addition of exogenous TGF-alpha. Targeting of Stat3 using antisense **oligonucleotides** directed against the translation initiation site, resulted in significant growth inhibition. In addition, cells stably transfected with dominant negative mutant Stat3 constructs failed to proliferate in vitro. In contrast, targeting of Stat1 using either antisense or dominant-negative strategies had no effect on cell growth. Thus, TGF-alpha/EGFR-mediated autocrine growth of transformed epithelial cells is dependent on activation of Stat3 but not Stat1.

7/3,AB/3 (Item 3 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09945762 98380997 PMID: 9715265

Characterization of cytokine differential induction of STAT complexes in primary human T and NK cells.

Yu C R; Young H A; Ortaldo J R

Division of Basic Sciences, National Cancer Institute, Frederick Cancer

Research and Development Center, National Institutes of Health, Frederick, Maryland 21702-1201, USA.

Journal of leukocyte biology (UNITED STATES) Aug 1998, 64 (2)
p245-58, ISSN 0741-5400 Journal Code: 8405628

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Cytokines, IL-2, IL-4, IL-6, IL-7, IL-12, and IL-15 are key regulators of human peripheral blood T and NK cell activation and differentiation but the precise mechanisms that give rise to their differential activities within these cells are not clear. Recent studies reveal that a family of **transcription** factors, signal transducers and activators of **transcription** (STATs) directly mediate many cytokine signals. We analyzed the activation of STATs in primary human T and NK cells by a variety of specific cytokines. We demonstrate that IL-12 induces STAT4 only in freshly isolated primary NK cells, but not in primary T cells, consistent with the lack of the IL-12 receptor in resting T cells. In contrast, IL-4 induces different C epsilon GAS DNA-protein binding complexes in both T and NK cells. Moreover, IL-4 costimulation with IL-2 or IL-12 does not alter their own preferential GAS-like DNA binding patterns when C epsilon-, Fc gamma RI-, and SIE GAS motif containing **oligonucleotide** probes are compared, suggesting that induction of GAS-like DNA-protein binding complexes by IL-2, IL-4, and IL-12 is highly selective and represents one important **factor** in determining specific gene activation. In addition, IL-6 and IL-2 synergistically induce homo- and heterodimerized STAT1 alpha and STAT3 in both NK and T cells, consistent with their reported synergism in modulating perforin gene expression. We further demonstrated that IL-2, -7, and -15 induce multiple **STAT** proteins, including STAT5a, STAT5b, STAT1 alpha, STAT3, and another unidentified Fc gamma RI GAS DNA-binding protein. Finally, we observed that activated STAT5a and STAT5b proteins form distinct Fc gamma RI GAS binding patterns in T and NK cells, suggesting that they might have different roles in gene regulation. Our data provide evidence that the differential responses in gene expression and cell activation seen in primary NK and T cells on direct stimulation with different cytokines may be a direct result of distinct activation of **STAT transcription** factors.

7/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09923070 98361782 PMID: 9694725

Differential binding activity of the **transcription factor** LIL-STAT in immature and differentiated normal and leukemic myeloid cells.

Tuyt L M; Bregman K; Lummen C; Dokter W H; Vellenga E
Division of Hematology and Center for Biomedical Technology, University of Groningen, The Netherlands.

Blood (UNITED STATES) Aug 15 1998, 92 (4) p1364-73, ISSN 0006-4971 Journal Code: 7603509

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Cytokines and growth factors induce activation of the family of signal transducers and activators of **transcription** (Stats) that directly activate gene expression. Recently, constitutively activated Stat1, Stat3, and Stat5 were identified in nuclear extracts of acute myeloid leukemia (AML) patients, suggesting involvement of constitutive **Stat** activity in the events of leukemogenesis. In the present study, blasts of nine AML cases were investigated for the constitutive binding activity of the recently identified **transcription factor** LIL-Stat (LPS-

and IL-1-inducible Stat). Band-shift assays were performed using the LPS-and IL-1-responsive element (LILRE) **oligonucleotide**, a gamma interferon activation site-like site that is present in the human IL-1beta promoter. Constitutive LIL-Stat binding activity was observed in three leukemic cell lines and in seven out of nine AML cases. Transient transfection studies with a reporter plasmid containing three sequential LIL-Stat binding sites showed distinct transcriptional activity of LIL-Stat only in those AML blasts that constitutively expressed LIL-Stat. In CD34+ cells LIL-Stat also constitutively bound to its consensus sequence. However, when these cells were cultured in the presence of macrophage-colony stimulating **factor** (M-CSF) and stem cell **factor** (SCF) for differentiation along the monocytic lineage, the LIL-Stat binding activity disappeared totally. In agreement with these findings neither mature monocytes nor granulocytes showed constitutive or inducible LIL-Stat binding activity. We conclude that the LIL-Stat **transcription factor** is constitutively activated in undifferentiated and leukemic hematopoietic cells, but not in mature cells. This may suggest a role for this **transcription factor** in the process of differentiation. Copyright 1998 by The American Society of Hematology.

7/3,AB/5 (Item 5 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09796486 98222613 PMID: 9561800

Lack of 2',5'-oligoadenylate-dependent RNase expression in the human hepatoma cell line HepG2.

Tnani M; Bayard B A

UMR 5539 Centre National de la Recherche Scientifique, Universite de Montpellier II, France.

Biochimica et biophysica acta (NETHERLANDS) Mar 27 1998, 1402

(2) p139-50, ISSN 0006-3002 Journal Code: 0217513

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

2',5'-adenylate **oligonucleotide** (2-5A)-dependent RNase and 2-5A-synthetase are two enzymes of the 2-5A system strongly implicated in the basal control of RNA decay of both interferon-treated and untreated cells. RNase is activated by a 2-5A produced by 2-5A-synthetase, both enzymes being overexpressed by type I-interferon (alpha/beta). We described here for the first time a cell line completely deficient in RNase and its mRNA, while p69 2-5A-synthetase was normally interferon alpha/beta-induced. The complete absence of this RNase in human hepatoma cells (HepG2) was shown using three different methods based on the binding of a [32P]-labeled 2-5A probe of high specific activity to its binding site. Negative Western blotting assay with a specific monoclonal antibody correlated the previous findings. RNase-specific mRNA was not detectable even after treatment of cells with 1000 units/ml of interferon alpha/beta. This is not due to a mutation of the gene because an intronless genomic DNA sequence encoding 2-5A-binding site was cloned and expressed. It is likely that the expression of 2-5A-dependent RNase was impaired at the transcriptional level while having the known IFN alpha/beta-transcriptional regulatory factors as revealed by induction of p69 2-5A-synthetase gene. This may account for a differential activation of 2-5A-dependent RNase and 2-5A-synthetase genes by type I-interferon, and suggests that other members of regulatory **transcription factors**, different from IRF-1 and **STAT** proteins, may participate in two different interferon alpha/beta signaling pathways.

7/3,AB/6 (Item 6 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09767022 98208267 PMID: 9548487

IL-4-induced STAT6 suppresses IFN-gamma-stimulated STAT1-dependent **transcription** in mouse macrophages.

Ohmori Y; Hamilton T A

Department of Immunology, The Cleveland Clinic Foundation, OH 44195, USA.

Journal of immunology (Baltimore, Md. : 1950) (UNITED STATES) Dec 1 1997, 159 (11) p5474-82, ISSN 0022-1767 Journal Code: 2985117R

Contract/Grant No.: CA39621; CA; NCI; CA62220; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

IL-4 suppresses the IFN-gamma-induced expression of the IFN regulatory **factor** -1 (IRF-1) gene, and this suppression is attenuated by increasing the amount of IFN-gamma. The effects of IFN-gamma and IL-4 on **transcription** of a reporter gene under control of a 1.3-kb fragment from the IRF-1 gene promoter or the **STAT** binding element (SBE) from this gene in the context of a heterologous promoter are similar to their effects on the endogenous IRF-1 gene. IFN-gamma-dependent **transcription** of reporter gene is suppressed by IL-4, but IL-4 alone has no trans-activating function. IL-4 treatment does not inhibit the tyrosine phosphorylation or nuclear translocation of IFN-gamma-activated STAT1. Rather, IFN-gamma and IL-4 independently activate STAT1 and STAT6, respectively, and both proteins bind to the IRF-1 SBE in homodimeric form. The affinity of STAT1 for the IRF-1 SBE is higher than the affinity of STAT6, as measured by competition with unlabeled **oligonucleotide**. These observations suggest that IL-4 may suppress IFN-gamma-stimulated **transcription** of the IRF-1 gene by activation of STAT6, which can compete with STAT1 for occupancy of the IRF-1 SBE when STAT1 levels are low. Suppression may be attenuated as the quantity of STAT1 relative to that of STAT6 increases in cells treated with increasing amounts of IFN-gamma and displaces STAT6.

7/3,AB/7 (Item 7 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

09674809 98101642 PMID: 9440692

Induction of epithelial tubules by growth **factor** HGF depends on the **STAT** pathway.

Boccaccio C; Ando M; Tamagnone L; Bardelli A; Michieli P; Battistini C; Comoglio P M

Institute for Cancer Research, University of Torino Medical School, Candiolo, Italy. cboccaccio@hal.ircc.polito.it

Nature (ENGLAND) Jan 15 1998, 391 (6664) p285-8, ISSN 0028-0836 Journal Code: 0410462

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Hepatocyte growth **factor** (HGF) induces a three-phase response leading to the formation of branched tubular structures in epithelial cells. The HGF receptor tyrosine kinase works through a Src homology (SH2) docking site that can activate several signalling pathways. The first phase of the response (scattering), which results from cytoskeletal reorganization, loss of intercellular junctions and cell migration, is dependent on phosphatidylinositol-3-OH kinase and Rac activation. The second phase (growth) requires stimulation of the Ras-MAP kinase cascade. Here we show that the third phase (tubulogenesis) is dependent on the **STAT** pathway. HGF stimulates recruitment of **Stat**-3 to the receptor, tyrosine phosphorylation, nuclear translocation and binding to the specific promoter element SIE. Electroporation of a tyrosine-phosphorylated peptide, which interferes with both the association

of **STAT** to the receptor and **STAT** dimerization, inhibits tubule formation in vitro without affecting either HGF-induced 'scattering' or growth. The same result is obtained using a specific 'decoy' oligonucleotide that prevents **STAT** from binding to DNA and affecting the expression of genes involved in cell-cycle regulation (c-fos and waf-1). Activation of signal transducers that directly control transcription is therefore required for morphogenesis.

7/3,AB/8 (Item 8 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09633103 98054332 PMID: 9391124

Proliferation of adult T cell leukemia/lymphoma cells is associated with the constitutive activation of JAK/**STAT** proteins.

Takemoto S; Mulloy J C; Cereseto A; Migone T S; Patel B K; Matsuoka M; Yamaguchi K; Takatsuki K; Kamihira S; White J D; Leonard W J; Waldmann T; Franchini G

Basic Research Laboratory, Division of Basic Sciences, National Cancer Institute, Bethesda, MD 20892, USA.

Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Dec 9 1997, 94 (25) p13897-902, ISSN 0027-8424 Journal Code: 7505876

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Human T cell leukemia/lymphotropic virus type I (HTLV-I) induces adult T cell leukemia/lymphoma (ATLL). The mechanism of HTLV-I oncogenesis in T cells remains partly elusive. In vitro, HTLV-I induces ligand-independent transformation of human CD4+ T cells, an event that correlates with acquisition of constitutive phosphorylation of Janus kinases (JAK) and signal transducers and activators of transcription (**STAT**) proteins. However, it is unclear whether the in vitro model of HTLV-I transformation has relevance to viral leukemogenesis in vivo. Here we tested the status of JAK/**STAT** phosphorylation and DNA-binding activity of **STAT** proteins in cell extracts of uncultured leukemic cells from 12 patients with ATLL by either DNA-binding assays, using DNA oligonucleotides specific for **STAT**-1 and **STAT**-3, **STAT**-5 and **STAT**-6 or, more directly, by immunoprecipitation and immunoblotting with anti-phosphotyrosine antibody for JAK and **STAT** proteins. Leukemic cells from 8 of 12 patients studied displayed constitutive DNA-binding activity of one or more **STAT** proteins, and the constitutive activation of the JAK/**STAT** pathway was found to persist over time in the 2 patients followed longitudinally. Furthermore, an association between JAK3 and **STAT**-1, **STAT**-3, and **STAT**-5 activation and cell-cycle progression was demonstrated by both propidium iodide staining and bromodeoxyuridine incorporation in cells of four patients tested. These results imply that JAK/**STAT** activation is associated with replication of leukemic cells and that therapeutic approaches aimed at JAK/**STAT** inhibition may be considered to halt neoplastic growth.

7/3,AB/9 (Item 9 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09623253 98042127 PMID: 9374714

Differential activation of the Stat signaling pathway in the liver after burn injury.

Wang S; Wolf S E; Evers B M

Department of Surgery, University of Texas Medical Branch, Galveston 77555, USA.

American journal of physiology (UNITED STATES) Nov 1997, 273 (5

Pt 1) pG1153-9, ISSN 0002-9513 Journal Code: 0370511

Contract/Grant No.: PO1 DK-35608; DK; NIDDK

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The liver plays a crucial role in the acute phase response after injury; mechanisms responsible for transducing inflammatory signals to the nucleus to initiate this response are not known. The purpose of this study was to examine the induction of the novel **Stat** (signal transducer and activator of **transcription**) pathway in the liver after burn injury. Rats were subjected to either a 60% burn or sham treatment; livers were removed over a time course and extracted for nuclear protein. We found that Stat3, but not Stat5, binding was predominantly increased in the liver after burn injury as assessed by gel mobility and "supershift" analyses. Moreover, Stat3 nuclear protein levels were increased 6- to 14-fold in the livers of burned rats compared with those of sham rats. Stat3 phosphorylation was rapidly induced after burn injury; the subsequent increase of Stat3 binding was completely blocked by preincubation with the antiphosphotyrosine antibody (4G10). We conclude that a differential and early induction of Stat3 binding activity occurs in the liver after burn injury; this induction is mediated by an increase in phosphorylation. These findings suggest an important role for Stat3 in transducing inflammatory signals to the nucleus of liver cells after a systemic burn injury.

7/3,AB/10 (Item 10 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09568596 97454445 PMID: 9355737

Stat1 associates with c-kit and is activated in response to stem cell **factor**.

Deberry C; Mou S; Linnekin D

Laboratory of Leukocyte Biology, Division of Basic Sciences, National Cancer Institute, USA.

Biochemical journal (ENGLAND) Oct 1 1997, 327 (Pt 1) p73-80,
ISSN 0264-6021 Journal Code: 2984726R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Interaction of stem cell **factor** (SCF), a haematopoietic growth **factor**, with the receptor tyrosine kinase c-kit leads to autophosphorylation of c-kit as well as tyrosine phosphorylation of various substrates. Little is known about the role of the JAK/**STAT** pathway in signal transduction via receptor tyrosine kinases, although this pathway has been well characterized in cytokine receptor signal transduction. We recently found that the Janus kinase Jak2 associates with c-kit and that SCF induces rapid and transient phosphorylation of Jak2. Here we present evidence that SCF activates the **transcription factor** Stat1. Phosphorylated c-kit co-immunoprecipitates with Stat1 within 1 min of SCF stimulation of the human cell line MO7e. Co-precipitation experiments using glutathione S-transferase fusion proteins indicate that association with c-kit is mediated by the Stat1 SH2 domain. Stat1 is rapidly tyrosine-phosphorylated in response to SCF in MO7e cells, the murine cell line FDCP-1 and normal progenitor cells. SCF-induced phosphorylation of Jak2 and Stat1 was also observed in murine 3T3 fibroblasts stably transfected with full-length human c-kit receptor. Furthermore c-kit directly phosphorylates Stat1 fusion proteins in in vitro kinase assays. Electrophoretic mobility-shift assays with nuclear extracts from SCF-stimulated cell lines and normal progenitor cells indicate that activated Stat1 binds the m67 **oligonucleotide**, a high-affinity SIE promoter sequence. These results demonstrate that Stat1 is activated in response to SCF, and suggest that Stat1 is a component of the SCF

signal-transduction pathway.

7/3,AB/11 (Item 11 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09443113 97342492 PMID: 9199189
Novel pathway of insulin signaling involving Stat1alpha in Hep3B cells.
Chuang L M; Wang P H; Chang H M; Lee S C
Department of Internal Medicine, College of Medicine, National Taiwan
University, Taipei.
Biochemical and biophysical research communications (UNITED STATES) Jun
18 1997, 235 (2) p317-20, ISSN 0006-291X Journal Code: 0372516
Contract/Grant No.: HL-55533; HL; NHLBI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

STAT proteins are important **transcription** factors that regulate cell growth and differentiation. To elucidate the molecular mechanisms of insulin actions, we have studied how insulin activates **STAT** proteins in Hep3B cells. Insulin rapidly phosphorylated Stat1alpha at tyrosine residues and increased its specific binding activities to a GAS/ISRE consensus **oligonucleotide**. IL-4 also phosphorylated Stat1alpha and increased DNA binding activities to the same Stat1alpha responsive element. There was no increase in tyrosine phosphorylation of JAK family of kinases following insulin stimulation. In contrast, IL-4 stimulated tyrosine phosphorylation of JAK1, JAK2 and tyk2 in this cell line. These data indicate that insulin receptor signaling can activate the transcriptional regulatory function of **STAT** protein, and that insulin actions on Stat1alpha are mediated through signaling pathways independent of JAK family of kinases.

7/3,AB/12 (Item 12 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09437720 97341191 PMID: 9195960
A distinct function of **STAT** proteins in erythropoietin signal transduction.
Kirito K; Uchida M; Yamada M; Miura Y; Komatsu N
Division of Hematology, Department of Medicine, Jichi Medical School,
Tochigi 329-04, Japan.
Journal of biological chemistry (UNITED STATES) Jun 27 1997, 272
(26) p16507-13, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The Janus kinase (JAK)-signal transducers and activators of **transcription** (**STAT**) pathway is an important signaling pathway of interferons and cytokines. We examined the activation of **STAT** proteins induced by interleukin-3 (IL-3), granulocyte-macrophage colony-stimulating factor (GM-CSF), or erythropoietin (EPO) using the human leukemia cell line, UT-7, which requires these cytokines for growth. IL-3, GM-CSF, and EPO induced DNA-binding activity to the **oligonucleotides** corresponding to the sis-inducible elements (SIE) of c-fos, in addition to the beta-casein promoter (beta-CAP), SIE- and beta-CAP-binding proteins were identical to Stat1alpha and Stat3 complex and to Stat5 protein, respectively. This indicates that IL-3, GM-CSF, and EPO commonly activated Stat1alpha, Stat3, and Stat5 proteins in UT-7. However, EPO hardly activated Stat1alpha and Stat3 in UT-7/GM, which is a subline of UT-7 that grows slightly in response to EPO. Transfection studies revealed that UT-7/GM cells constitutively expressing Stat1alpha,

but not Stat3, can grow as well in response to EPO as GM-CSF, suggesting that Stat1alpha is involved in the EPO-induced proliferation of UT-7. Thus, although Stat1alpha, Stat3, and Stat5 proteins are activated by GM-CSF, IL-3, and EPO, our data suggest that each **STAT** protein has a distinctive role in the actions of cytokines.

7/3,AB/13 (Item 13 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09427593 97318687 PMID: 9175624

Induction of Sp1 activity by prolactin and interleukin-2 in Nb2 T-cells: differential association of Sp1-DNA complexes with Stats.

Too C K

Department of Biochemistry, Dalhousie University, Halifax, Nova Scotia, Canada. CTOO@IS.DAL.CA

Molecular and cellular endocrinology (IRELAND) Apr 25 1997, 129

(1) p7-16, ISSN 0303-7207 Journal Code: 7500844

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The induction of the **transcription factor** Sp1 by prolactin (PRL) and interleukin-2 (IL-2) was investigated in the PRL- and IL-2 responsive rat Nb2 T-cell line. Western analysis showed a rapid increase in Sp1 synthesis in Nb2 cells in response to PRL or IL-2. Elevation of Sp1 protein levels occurred within 15 min following PRL or IL-2 stimulation, reached a maximum by 1 h and was inhibited by cycloheximide, indicating de novo protein synthesis. Interestingly, dilution of confluent, growth-arrested Nb2 cells to low density also caused a rapid elevation in Sp1 suggesting that growth arrest may down-regulate Sp1 synthesis. Electrophoretic mobility shift assays using an Sp1 consensus **oligonucleotide** as probe showed a rapid but transient formation of a single PRL-inducible complex at 30 min. In contrast, three IL-2-inducible complexes were formed at 30 min and persisted to at least 60 min. Mobility shift interference assays using specific **Stat** antibodies failed to detect Stat1alpha, Stat3 or Stat5 in the 30 min PRL-inducible complex. In contrast, the IL-2 induced complexes contained Stat3 alone at 30 min and both Stat3 and Stat5 at 60 min. The PRL- and IL-2-inducible complexes did not contain the tumor suppressor protein, p53. The time dependent association of the **Stat** proteins with the IL-2-inducible complexes, but not with the PRL-inducible complex, suggests that the two mitogens may selectively utilize specific promoter elements for transcriptional activation of PRL- and IL-2-responsive genes. Alternatively, the two mitogens may be activating different genes with Sp1-binding promoter elements for their mitogenic action in Nb2 cells.

7/3,AB/14 (Item 14 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09413046 97306310 PMID: 9162035

Prolactin stimulates serine/tyrosine phosphorylation and formation of heterocomplexes of multiple Stat5 isoforms in Nb2 lymphocytes.

Kirken R A; Malabarba M G; Xu J; Liu X; Farrar W L; Hennighausen L; Larner A C; Grimley P M; Rui H

Intramural Research Support Program, Science Applications International Corporation Frederick, Frederick, Maryland 21702-1201, USA.

Journal of biological chemistry (UNITED STATES) May 30 1997, 272

(22) p14098-103, ISSN 0021-9258 Journal Code: 2985121R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Transcription factors of the **Stat** gene family are selectively activated by many hormones and cytokines. Stat5 originally was cloned as a prolactin-stimulated DNA-binding protein, but is also activated by non-lactogenic cytokines in many cell types. The recent identification of two distinct Stat5 genes, which encode a 94-kDa Stat5a and a 92-kDa Stat5b as well as several lower molecular weight isoforms, suggests additional complexity and combinatorial possibilities for transcriptional regulation. We now report a biochemical analysis of prolactin activation of **Stat** proteins in Nb2 lymphocytes, which was associated with: 1) rapid tyrosine phosphorylation of Stat5a, Stat5b, a COOH-terminally truncated 80-kDa Stat5 form, Stat1alpha, and Stat3; 2) rapid and selective formation of Stat5a/b heterodimers, without involvement of Stat1alpha or Stat3; 3) marked serine, but not threonine phosphorylation of Stat5a and Stat5b; and 4) the appearance of two qualitatively distinct Stat5 protein complexes, which discriminated between **oligonucleotides** corresponding to the prolactin response elements of the beta-casein and interferon regulatory **factor** -1 gene promoters. Collectively, our analyses showed that Stat5a and Stat5b respond similarly to prolactin receptor activation, but also suggested that the two genes have evolved unique properties that may contribute to the specificity of receptors that utilize Stat5 signaling proteins.

7/3,AB/15 (Item 15 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09384337 97289504 PMID: 9144409

Binding sequence of STAT4: STAT4 complex recognizes the IFN-gamma activation site (GAS)-like sequence (T/A)TTCC(C/G)GGAA(T/A).

Yamamoto K; Miura O; Hirosawa S; Miyasaka N

The First Department of Internal Medicine, Tokyo Medical and Dental University, Bunkyo-ku, Japan.

Biochemical and biophysical research communications (UNITED STATES) Apr 7 1997, 233 (1) p126-32, ISSN 0006-291X Journal Code: 0372516

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Studies of transcriptional activation by interferons and various cytokines have led to the identification of a family of proteins that serve as signal transducers and activators of **transcription (STAT)**.

STAT4 is phosphorylated following interleukin (IL)-12 stimulation and is required for IL-12 signal transduction. By immunoprecipitation and PCR amplification, a specific consensus sequence for DNA binding of the STAT4 complex was determined. The binding sequence of the STAT4 complex, (T/A)TTCC(C/G)GGAA(T/A), proved to be palindromic and similar to the IFN-gamma activated site (GAS)-like sequence. The first (T/A) and last (T/A) sites of the consensus sequence were critical for the binding affinity of the STAT4 complex.

7/3,AB/16 (Item 16 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09376700 97277628 PMID: 9131012

Stem cell **factor** activates **STAT**-5 DNA binding in IL-3-derived bone marrow mast cells.

Ryan J J; Huang H; McReynolds L J; Shelburne C; Hu-Li J; Huff T F; Paul W E

Laboratory of Immunology, National Institute of Allergy and Infectious Disease, NIH, Bethesda, MD 20892-1892, USA.

Experimental hematology (UNITED STATES) Apr 1997, 25 (4) p357-62, ISSN 0301-472X Journal Code: 0402313

Contract/Grant No.: R01AI25537; AI; NIAID

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The Kit tyrosine kinase regulates growth and differentiation of many hematopoietic cells through a signal transduction process that remains to be fully elucidated. Kit has been shown to associate with the receptor for erythropoietin (Epo), which is known to activate the signal transducer and activator of **transcription**, **STAT-5**. To determine if Kit signal transduction activated latent DNA-binding factors, including **STAT-5**, we performed electrophoretic mobility shift assays on stem cell **factor** (SCF)-stimulated mouse bone marrow-derived mast cells (BMMCs). SCF led to the rapid and transient activation of a DNA-binding **factor** that was identified by supershift analysis as **STAT-5**. **STAT-5** DNA binding was shown to be specific for the **oligonucleotide** of the correct sequence and was dose-responsive. Epo stimulation of BMMCs led to the activation of a DNA-binding activity that comigrated with the SCF-induced band, but peaked and was maintained at later time points than SCF-induced activation. These data indicate that SCF stimulation of Kit leads to activation of **STAT-5** DNA binding with kinetics distinct from Epo-mediated stimulation.

7/3,AB/17 (Item 17 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

09165890 97066996 PMID: 8910398

Stat3 recruitment by two distinct ligand-induced, tyrosine-phosphorylated docking sites in the interleukin-10 receptor intracellular domain.

Weber-Nordt R M; Riley J K; Greenlund A C; Moore K W; Darnell J E; Schreiber R D

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Journal of biological chemistry (UNITED STATES) Nov 1 1996, 271

(44) p27954-61, ISSN 0021-9258 Journal Code: 2985121R

Contract/Grant No.: CA43059; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Recent work has shown that IL-10 induces activation of the JAK-**STAT** signaling pathway. To define the mechanism underlying signal transducer and activator of **transcription** (**STAT**) protein recruitment to the interleukin 10 (IL-10) receptor, the **STAT** proteins activated by IL-10 in different cell populations were first defined using electrophoretic mobility shift assays. In all cells tested, IL-10 activated Stat1 and Stat3 and induced the formation of three distinct DNA binding complexes that contained different combinations of these two **transcription** factors. IL-10 also activated Stat5 in Ba/F3 cells that stably expressed the murine IL-10 receptor. Using a structure-function mutagenesis approach, two tyrosine residues (Tyr427 and Tyr477) in the intracellular domain of the murine IL-10 receptor were found to be redundantly required for receptor function and for activation of Stat3 but not for Stat1 or Stat5. Twelve amino acid peptides encompassing either of these two tyrosine residues in phosphorylated form coprecipitated Stat3 but not Stat1 and blocked IL-10-induced Stat3 phosphorylation in a cell-free system. In contrast, tyrosine-phosphorylated peptides containing Tyr374 or Tyr396 did not interact with Stat3 or block Stat3 activation. These data demonstrate that Stat3 but not Stat1 or Stat5 is directly recruited to the ligand-activated IL-10 receptor by binding to specific but redundant receptor intracellular domain sequences containing phosphotyrosine. This study thus supports the concept that utilization of distinct **STAT** proteins by different cytokine receptors is dependent on the expression of particular ligand-activatable, tyrosine-containing **STAT** docking sites in

receptor intracellular domains.

7/3,AB/18 (Item 18 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09049475 96422801 PMID: 8825418

Host genotype controls the ability of the ISGF3 complex to activate **transcription** of IFN-inducible genes.

Gariglio M; Foresta P; Ying G G; Gaboli M; Lembo D; Landolfo S

Institute of Microbiology, University of Torino, Italy.

Journal of cellular biochemistry (UNITED STATES) Jan 1996, 60

(1) p83-94, ISSN 0730-2312 Journal Code: 8205768

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

C57BL/6 mice are unable to express the Ifi 202 type genes upon injection in vivo of multiple dsRNA, poly rI:rC, or IFN-treatment in vitro. For this purpose the 5' terminal flanking region (called the b segment of 804 bp) was linked to a heterologous reporter gene chloramphenicol acetyl transferase (CAT) and transfected into NIH3T3 cells or BLK cells derived from the C57BL/6 strain. IFN-alpha induced strong CAT activity in NIH3T3 but not in BLK cells. This lack of **transcription** activation was not due to a defect in **STAT factor** activity, since IFN-alpha treatment in the presence of IFN-gamma priming induced translocation of the ISGF3 into the nucleus, and binding to the ISRE (IFN-Stimulated Response Element) of the 202 gene even in C57BL/6 derived cells. Surprisingly when three tandem copies of the 202 ISRE (42 bp) were linked to a heterologous promoter (c-fos promoter) driving the reporter CAT gene, activation was also observed in C57BL/6 cells upon IFN-treatment. Finally, another IFN-inducible gene, namely the Mx, was activated in C57BL/6 mice. Thus, the primary defect of the C57BL/6 strain leading to an impaired Ifi 202 type gene response to IFN appears to be an inability of the ISGF3 complex to activate the endogenous promoter. Altogether these results suggest that unidentified nuclear factors related to the host genotype control the ability of the **STAT** factors to activate **transcription** upon IFN-treatment.

7/3,AB/19 (Item 19 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08998141 96354825 PMID: 8753801

STAT-like DNA-binding activity in Spodoptera frugiperda cells.

Sliva D; Haldosen L A

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Biochemical and biophysical research communications (UNITED STATES) Aug 14 1996, 225 (2) p562-9, ISSN 0006-291X Journal Code: 0372516

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Nuclear extracts from Spodoptera frugiperda (Sf9) cells were shown to contain a **factor** binding to DNA elements with gamma interferon activated site-like sequences. The DNA-binding activity was shown to be dependent on tyrosine phosphorylation. Hydrodynamic characterization of this entity revealed a Stokes radius of 8.4 nm and a sedimentation coefficient of 5.9 S. The molecular weight was calculated to 209,000. Estimated frictional (f/to) and axial (a/b) ratios indicated an elongated structure of this DNA-binding entity. This DNA-binding **factor** could represent a dimer of a Sf9 homolog to the mammalian signal transducers and activators of **transcription**.

7/3,AB/20 (Item 20 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08969533 96311205 PMID: 8732682

Characterization and cloning of STAT5 from IM-9 cells and its activation by growth hormone.

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Department of Medicine, University of Virginia Health Sciences Center,
Charlottesville 22908, USA.

Molecular endocrinology (Baltimore, Md.) (UNITED STATES) May 1996

, 10 (5) p508-18, ISSN 0888-8809 Journal Code: 8801431

Contract/Grant No.: DK-43701; DK; NIDDK; DK-48481; DK; NIDDK

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The interaction of GH with its receptor has been shown to lead to the phosphorylation of the signal transducer and activator of **transcription** (STAT) family of **transcription** factors. We demonstrate here that GH activates the tyrosine phosphorylation of STAT5 in the human IM-9 lymphocyte cell line. Western blotting indicates that GH also activates STAT5 in human embryonic kidney cells (293), which stably express the rabbit GH receptor. Although it has been shown previously that GH activates both STATs 1 and 3 in the 3T3-F442A mouse preadipocyte cell line, we demonstrate that GH also activates STAT5 in these cells. Using electrophoretic mobility shift assay, we examined the interaction of proteins with DNA elements containing consensus **STAT**-binding sequences. Proteins prepared from GH-treated 3T3-F442A cells bound to the c-sis inducible element of the human c-fos gene (m67 SIE), whereas proteins from GH-treated IM-9 cells did not. However, proteins from GH-treated IM-9 cells did interact with **oligonucleotides** containing either an interferon response element or the lactogenic hormone-responsive region. Treatment of IM-9 cells with interferon-gamma also induced protein interactions with these elements although the complexes were distinctly different than those seen with GH treatment. Using **STAT**-specific antibodies, we demonstrate that the GH-induced DNA-protein complex formed with the lactogenic hormone-responsive region contained STAT5, while the interferon-gamma-induced complex contained STAT1. These results implicate STAT5 as a downstream mediator of GH action in IM-9 cells. We report here the cloning of two forms of STAT5, STAT5A and STAT5B, from an IM-9 cDNA library. Northern blot analysis demonstrated multiple-forms of STAT5 mRNA in IM-9 cells.

7/3,AB/21 (Item 21 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08959998 96309621 PMID: 8704235

Constitutive activation of **STAT** proteins in primary lymphoid and myeloid leukemia cells and in Epstein-Barr virus (EBV)-related lymphoma cell lines.

Weber-Nordt R M; Egen C; Wehinger J; Ludwig W; Gouilleux-Gruart V;
Mertelsmann R; Finke J

Department of Hematology & Oncology, Albert-Ludwigs-University Medical
Center, Freiburg, Germany.

Blood (UNITED STATES) Aug 1 1996, 88 (3) p809-16, ISSN

0006-4971 Journal Code: 7603509

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Although various molecular mechanisms of **STAT** protein (signal transducers and activators of **transcription**) activation have been

identified, little is known about the functional role of **STAT**-dependent transcriptional activation. Herein we report the constitutive nuclear localization, phosphorylation, and DNA-binding activity of **STAT** proteins in leukemia cells and lymphoma cell lines. With the use of **oligonucleotide** probes derived from the Fc gamma RI promoter, the beta-casein promoter and a **STAT**-binding element in the promoter of the Bcl-2 gene constitutive activation of **STAT** proteins was detected in untreated acute T- and C/B-leukemia cells (3 of 5 and 12 of 19 patients, respectively). Supershift analyses using Stats 1-6 specific antisera showed the constitutive DNA binding activity of Stat5 in these cells. Confocal microscopy revealed the nuclear localization of Stat5 and Western blot analyses showed tyrosine phosphorylation of Stat5 in nuclear extracts of acute leukemia cells. In contrast, peripheral blood mononuclear cells did not display constitutive **STAT**-DNA interaction. Further studies were performed on freshly isolated acute myeloid leukemia cells as well as on cell line derived K562, lymphoblastoid cells (LCL), and Burkitt's lymphoma cells (BL). Fluorescence microscopy, gelshift, and supershift experiments showed the nuclear localization and constitutive DNA-binding activity of Stat5 in K562 cells. Stat1 and Stat3 were constitutively activated in freshly isolated AML cells (10 of 14 patients) and in Epstein Barr virus-positive or interleukin-10 expressing permanent LCL and BL cells. Thus, these data indicate a differential pattern of **STAT** protein activation in lymphoid or myeloid leukemia and in lymphoma cells.

7/3,AB/22 (Item 22 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08949154 96300436 PMID: 8737372

Prolactin recruits STAT1, STAT3 and STAT5 independent of conserved receptor tyrosines TYR402, TYR479, TYR515 and TYR580.

DaSilva L; Rui H; Erwin R A; Howard O M; Kirken R A; Malabarba M G; Hackett R H; Larner A C; Farrar W L

SAIC Frederick, National Cancer Institute, Frederick Cancer Research and Development Center, MD 21702, USA.

Molecular and cellular endocrinology (IRELAND) Mar 25 1996, 117

(2) p131-40, ISSN 0303-7207 Journal Code: 7500844

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The present study of prolactin (PRL) receptor-mediated recruitment of signal transducers and activators of **transcription** (STATs) demonstrates that PRL activates STAT3, in addition to STAT1 and STAT5 as previously reported, and that STAT1, STAT3 and STAT5 are mediators of PRL effects in cells whether of lymphoid, myeloid or mammary epithelial origin. Furthermore, receptor mutants M240 and T280 that do not mediate PRL-induced JAK2 activation and cell proliferation, are also unable to mediate **STAT** activation, supporting the proposed model of JAK2 as the initial effector protein used by PRL receptors. On the other hand, tyrosine phosphorylation analysis and electrophoretic mobility shift assays showed that receptor mutant G328, which lacks four of the five conserved cytoplasmic tyrosine residues of PRL receptors, retained the ability to activate JAK2 and STAT1, STAT3 and STAT5. These results support the notion that phosphotyrosyl residues other than those of the receptor, i.e., JAK2, are involved in recruiting **STAT** proteins to the activated PRL receptor complex.

7/3,AB/23 (Item 23 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08944738 96291549 PMID: 8730515

Uncoupling of the pathways which link MAP kinase to c-fos

transcription and AP-1 in response to growth stimuli.

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Beatson Institute for Cancer Research, CRC Beatson Laboratories, Bearsden, UK.

Cellular signalling (ENGLAND) Feb 1996, 8 (2) p131-9, ISSN 0898-6568 Journal Code: 8904683

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The v-Src oncoprotein induces mitogenesis and transformation of cells through multiple effects on diverse signalling pathways that are influenced by the cellular context in which v-Src is expressed. Here we have examined the effects of a temperature-sensitive (ts) v-Src on **transcription** of the c-fos proto-oncogene, in serum-deprived and growing Rat-1 fibroblasts. We have also considered the role of mitogen-activated protein (MAP) kinase, a known mediator of ternary complex formation at the c-fos serum response element (SRE), which results in transcriptional enhancement in response to growth factors. In cells exponentially growing in the presence of serum, activation of v-Src stimulated MAP kinase and c-fos **transcription**. In cells made quiescent by serum deprivation, however, v-Src did not induce a c-fos transcriptional response, nor was there stimulation of ternary complex formation, despite normal activation of MAP kinase. Thus, activation of MAP kinase and stimulation of c-fos **transcription** and ternary complex formation are uncoupled in the absence of serum growth factors. Stimulation of c-fos by v-Src in growing cells, however, coincided with formation of a complex with an **oligonucleotide** spanning the c-Sis-inducible element (SIE) upstream from the SRE, suggesting that the signal transduction and activator of **transcription** (STAT) family of **transcription** factors, which bind here, may function in response to the v-Src oncoprotein. During these studies, we also observed that addition of fresh serum growth factors to growing Rat-1 fibroblasts expressing ts v-Src at the restrictive temperature resulted in substantially impaired activation of MAP kinase. This interference with normal growth **factor** signalling implies that catalytically inactive Src acts in a dominant negative manner by blocking normal activation of MAP kinase, although not at the expense of c-fos **transcription**. Thus, serum-induced c-fos **transcription** can also occur in an MAP kinase-independent manner.

7/3,AB/24 (Item 24 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08938786 96284608 PMID: 8728040

Differential activation of the extracellular signal-regulated kinase, Jun kinase and Janus kinase-Stat pathways by oncostatin M and basic fibroblast growth **factor** in AIDS-derived Kaposi's sarcoma cells.

Faris M; Ensoli B; Stahl N; Yancopoulos G; Nguyen A; Wang S; Nel A E

Department of Medicine, University of California Los Angeles School of Medicine 90024, USA.

AIDS (London, England) (UNITED STATES) Apr 1996, 10 (4)
p369-78, ISSN 0269-9370 Journal Code: 8710219

Contract/Grant No.: 02104-15-RG; RG; CSR; CA09120-20; CA; NCI; CA6532901; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

OBJECTIVES: To determine the integration of signalling pathways associated with two recognized Kaposi's sarcoma (KS) growth factors, oncostatin M (OSM) and basic fibroblast growth **factor** (bFGF), in the induction of KS cell proliferation. DESIGN AND METHODS: We used protein kinase assays, protein-DNA interactions and AP-1 luciferase assays to study

the extracellular signal-regulated kinase (ERK), Janus kinase (JAK)-Stat and Jun kinase (JNK) pathways in AIDS-derived KS cells during stimulation with OSM and bFGF. RESULTS: Treatment with OSM-induced activation of receptor-associated JAK and phosphorylation of Stat1 and Stat3. Stat1/Stat3 heterodimers interacted with known gamma-interferon-activated sites like elements such as the sis-inducible element (SIE) in the C-fos promoter. In contrast, ligation of the bFGF receptor induced Stat3 phosphorylation and its association with the bFGF receptor, but failed to induce JAK activity or protein complexes which interact with GAS-like oligonucleotides. OSM also induced the activation of ERK2 by activating the serine/threonine kinases Raf-1 and [mitogenactivated protein kinase (MAPK) ERK kinase (MEK1)]-1, while bFGF failed to activate any of the above components. Both OSM and bFGF activated the JNK pathway, along with the activation of MEKkinase (MEKK)-1. JNK control the transcriptional activation of c-Jun. Because the above pathways exert an effect on the expression or activation of activation protein (AP)-1 components, we confirm that OSM and bFGF induce TPA response element (TRE)-luciferase activity synergistically. CONCLUSION: We demonstrate that OSM and bFGF activate distinct as well as shared signalling cascades in KS cells, which integrate to provide a synergistic AP-1 response by which OSM and bFGF may sustain KS cell growth.

7/3,AB/25 (Item 25 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08882428 96216570 PMID: 8660795

LPS does not directly induce **STAT** activity in mouse macrophages.
Deng W; Ohmori Y; Hamilton T A
Department of Immunology, Research Institute, Cleveland Clinic
Foundation, Ohio 44195, USA.
Cellular immunology (UNITED STATES) May 25 1996, 170 (1) p20-4
, ISSN 0008-8749 Journal Code: 1246405
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Induction of gene expression in cytokine-treated cells involves the protein tyrosine kinase-dependent activation of members of the **STAT** family of **transcription** factors. To determine if lipopolysaccharide (LPS) might activate one or more **STAT** factors, nuclear extracts from LPS-treated RAW264.7 macrophages were assayed for **STAT**-like DNA binding activity using oligonucleotides recognized by different members of this protein family. Within 30 min a single LPS-inducible DNA-protein complex was detected using three separate oligonucleotides. This activity was not reactive with anti-**STAT** antibodies and was subsequently identified as composed of the NF kappa B components NF kappa B1 and Rel-A. Thus, LPS does not directly stimulate **STAT** factors with known sequence-specific DNA binding activity.

7/3,AB/26 (Item 26 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08854430 96202489 PMID: 8634413

STAT-related **transcription** factors are constitutively activated in peripheral blood cells from acute leukemia patients.
Gouilleux-Gruart V; Gouilleux F; Desaint C; Claisse J F; Capiod J C; Delobel J; Weber-Nordt R; Dusanter-Fourt I; Dreyfus F; Groner B; Prin L
Laboratoire d'Immunologie, Centre Hospitalier Universitaire d'Amiens, France.
Blood (UNITED STATES) Mar 1 1996, 87 (5) p1692-7, ISSN 0006-4971 Journal Code: 7603509
Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

A signal transduction pathway activated by many cytokines has recently been elaborated. The JAK kinases and the signal transducers and activators of **transcription** (**STAT**) factors have been found to be essential components. In this report, we describe the presence of constitutively activated **STAT** factors in peripheral blood cells from patients with acute leukemia. We used **oligonucleotide** probes from the beta-casein and IRF-1 gene promoters and the ISRE probe to detect **STAT** proteins in nuclear extracts from acute leukemia cells in bandshift assays. Specific DNA protein complex formation was observed with the probes from the beta-casein and IRF-1 gene promoters, but not with the ISRE **oligonucleotide** probe, when cell extracts from acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) were investigated. We used nonradioactive **oligonucleotides** as competitors to show the specificity of the complex formation. Specific antibodies directed against the individual **STAT** proteins were used in supershift experiments. STAT5- and STAT1-related factors were detected in ALL and STAT1-, STAT3-, and STAT5-related proteins were present in nuclear cell extracts from AML. Since the cells were not treated with cytokines before the nuclear proteins were extracted, we conclude that these factors are constitutively activated in vivo. It is likely that the constitutive activation of **STAT** proteins is a part of the events of leukemogenesis.

7/3,AB/27 (Item 27 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08847062 96215064 PMID: 8621467

Growth hormone activation of **Stat 1**, **Stat 3**, and **Stat 5** in rat liver. Differential kinetics of hormone desensitization and growth hormone stimulation of both tyrosine phosphorylation and serine/threonine phosphorylation.

Ram P A; Park S H; Choi H K; Waxman D J

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Journal of biological chemistry (UNITED STATES) Mar 8 1996, 271

(10) p5929-40, ISSN 0021-9258 Journal Code: 2985121R

Contract/Grant No.: DK33765; DK; NIDDK

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Intermittent plasma growth hormone (GH) pulses, which occur in male but not female rats, activate liver **Stat 5** by a mechanism that involves tyrosine phosphorylation and nuclear translocation of this latent cytoplasmic **transcription factor** (Waxman, D. J., Ram, P. A., Park, S. H., and Choi, H. K. (1995) J. Biol. Chem. 270, 13262-13270). We demonstrate that physiological levels of GH can also activate **Stat 1** and **Stat 3** in liver tissue, but with a dependence on the dose of GH and its temporal plasma profile that is distinct from **Stat 5** and with a striking desensitization following a single hormone pulse that is not observed with liver **Stat 5**. GH activation of the two groups of **Stats** leads to their selective binding to DNA response elements upstream of the c-fos gene (c-sis-inducible enhancer element; **Stat 1** and **Stat 3** binding) and the beta-casein gene (mammary gland **factor** element; liver **Stat 5** binding). In addition to tyrosine phosphorylation, GH is shown to stimulate phosphorylation of these **Stats** on serine or threonine in a manner that either enhances (**Stat 1** and **Stat 3**) or substantially alters (liver **Stat 5**) the binding of each **Stat** to its cognate DNA response element. These findings establish the occurrence of multiple, **Stat**-dependent GH signaling pathways in liver cells that can target distinct genes and thereby contribute to the diverse effects

that GH and its sexually dimorphic plasma profile have on liver gene expression.

7/3,AB/28 (Item 28 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08839143 96190048 PMID: 8608222

The membrane-distal cytoplasmic region of human granulocyte colony-stimulating **factor** receptor is required for STAT3 but not STAT1 homodimer formation.

de Koning J P; Dong F; Smith L; Schelen A M; Barge R M; van der Plas D C; Hoefsloot L H; Lowenberg B; Touw I P

Institute of Hematology, Erasmus University, Rotterdam, The Netherlands.

Blood (UNITED STATES) Feb 15 1996, 87 (4) p1335-42, ISSN 0006-4971 Journal Code: 7603509

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Signal transduction from the granulocyte colony-stimulating **factor** receptor (G-CSF-R) involves the activation of the Janus tyrosine kinase/signal transducer and activator of **transcription** (Jak/STAT) pathway. G-CSF induces tyrosine phosphorylation of Jak1, Jak2, STAT1, and STAT3. The membrane-proximal region of G-CSF-R is sufficient for activation of Jaks. It is still unclear how **STAT** proteins are activated by G-CSF-R. We investigated the possible involvement of the C-terminal region of G-CSF-R in the recruitment of **STAT** proteins using BAF3 cell transfectants expressing wild type (WT) G-CSF-R, C-terminal deletion mutants and tyrosine-to-phenylalanine substitution mutants. Electrophoretic mobility shift assays with **STAT**-binding oligonucleotides (m67) showed that activation of WT G-CSF-R induces three distinct **STAT** complexes, namely STAT3 homodimers, STAT1-STAT3 heterodimers, and STAT1 homodimers. However, STAT1 homodimers and STAT1-STAT3 heterodimers were predominantly formed after activation of a C-terminal deletion mutant d685, which lacks all four conserved cytoplasmic tyrosine residues, located at positions 704, 729, 744, and 764. Antiphosphotyrosine immunoblots of STAT3 immunoprecipitates showed that activation of WT G-CSF-R induced phosphorylation of STAT3. In contrast, no phosphorylation of STAT3 was observed after activation of deletion mutant d685. These findings establish that the C-terminal region of G-CSF-R plays a major role in the activation of STAT3. By using tyrosine-to-phenylalanine substitution mutants of G-CSF-R, we further show that tyrosine 704, present in a YXXQ consensus sequence shown to be essential for STAT3 binding to gp130, is not exclusively involved in the activation of STAT3 by G-CSF-R.

7/3,AB/29 (Item 29 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08776271 96127783 PMID: 8541528

Granulocyte colony-stimulating **factor** rapidly activates a distinct **STAT**-like protein in normal myeloid cells.

Tweardy D J; Wright T M; Ziegler S F; Baumann H; Chakraborty A; White S M; Dyer K F; Rubin K A

Department of Medicine, University of Pittsburgh School of Medicine, PA, USA.

Blood (UNITED STATES) Dec 15 1995, 86 (12) p4409-16, ISSN 0006-4971 Journal Code: 7603509

Contract/Grant No.: AI07333; AI; NIAID; CA26122; CA; NCI; CA55333; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Binding of granulocyte colony-stimulating factor (G-CSF) to normal myeloid cells activates the protein tyrosine kinases Lyn and Syk and results in the immediate early upregulation of G-CSF receptor (R) mRNA. In our studies of the signaling pathways activated by G-CSF that are coupled to proliferation and differentiation of myeloid cells, we examined whether G-CSF activated a latent **transcription factor** belonging to the **STAT** protein family. Electrophoretic mobility shift assays (EMSAs) of nuclear extracts from G-CSF-stimulated human myeloid cells showed the rapid activation of a DNA-binding protein that bound to the high-affinity serum-inducible element (hSIE) and migrated with mobility similar to serum inducible factor (SIF)-A (Stat3 homodimer). The G-CSF-stimulated SIF-A complex (G-SIF-A) did not bind to duplex **oligonucleotides** used to purify and characterize other **Stat** proteins (Stat1-6). In addition, antibodies raised against Stat1-6 failed to supershift the G-SIF-A complex or interfere with its formation. Based on its binding to the hSIE and lack of antigenic cross-reactivity with other known **STAT** proteins that bind to this element, it is likely that G-SIF-A is composed of a distinct member of the **STAT** protein family. EMSAs of whole-cell extracts prepared from cell lines containing full-length and truncated mutants of the G-CSFR showed that activation of G-SIF-A did not correlate with proliferation; rather, optimal activation requires the distal half of the cytosolic domain of the G-CSFR that is essential for differentiation. Activation of G-SIF-A, therefore, may be an early G-CSFR-coupled event that is critical for myeloid maturation.

7/3,AB/30 (Item 30 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08719872 96062272 PMID: 7594456

Activation of the signal transducer and **transcription (STAT)** signaling pathway in a primary T cell response. Critical role for IL-6.

Henttinen T; Levy D E; Silvennoinen O; Hurme M

Department of Microbiology and Immunology, University of Tampere Medical School, Finland.

Journal of immunology (Baltimore, Md. : 1950) (UNITED STATES) Nov 15 1995, 155 (10) p4582-7, ISSN 0022-1767 Journal Code: 2985117R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The T cell activation is initiated by interaction of specific Ags with TCR, followed by activation of intracellular biochemical events leading to activation of several genes. The activation of signal transducer and activator of **transcription (STAT)** proteins in a primary TCR-mediated activation of T cells have been explored. In purified human peripheral blood T cells, nuclear **STAT** proteins were activated approximately 3 h after activation by cross-linked anti-CD3 Abs. These **STAT** proteins were detected by using the IFN-gamma-activated sequence (GAS) and related **oligonucleotides** as probes in electrophoretic mobility shift assay. Analysis of the nuclear extracts with anti-**STAT** Abs indicated that they contained **STAT-3** and additional proteins crossreactive with the **STAT** family. The induction of **STAT** activity was inhibited completely by pretreatment with either cycloheximide or cyclosporin A, thus indicating that the induction was due to a secondary **factor** produced by the activated T cells. As neutralizing anti-IL-6 Abs effectively down-regulated the early induction of **STAT** proteins and as exogenously added IL-6 rapidly activated DNA binding similar to TCR-mediated bindings, it can be concluded that IL-6 is the **factor** responsible for the activation of **STAT** proteins in a primary T cell response.

7/3,AB/31 (Item 31 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08698893 96033671 PMID: 7595475

Differences in nuclear signaling by leukemia inhibitory **factor** and interferon-gamma: the role of **STAT** proteins in regulating vasoactive intestinal peptide gene expression.

Symes A J; Corpus L; Fink J S

Department of Neurology, Massachusetts General Hospital, Boston 02114, USA.

Journal of neurochemistry (UNITED STATES) Nov 1995, 65 (5)
p1926-33, ISSN 0022-3042 Journal Code: 2985190R

Contract/Grant No.: NS27514; NS; NINDS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

To investigate the importance of **STAT** protein activation in leukemia inhibitory **factor** (LIF)-mediated induction of neuropeptide gene **transcription**, we compared signaling to the 180-bp cytokine response element (CyRE) in the vasoactive intestinal peptide (VIP) promoter by interferon-gamma (IFN-gamma) and LIF. We show that LIF and IFN-gamma activate **STAT** proteins but only LIF activates VIP gene **transcription**. Thus **STAT** activation is not sufficient for VIP transcriptional activation. In a CyRE reporter plasmid, in which the **STAT** site has been deleted, LIF, but not IFN-gamma, activates **transcription**, indicating that sequences within the CyRE distinct from the **STAT** site are important to LIF-mediated transcriptional activation. The CyRE does not mediate transcriptional activation to LIF in a non-VIPergic cell line, suggesting that cell-specific factors exist which are permissive for cytokine-dependent regulation of gene expression. Human and mouse sequences are highly conserved in the region of the CyRE, consistent with the functional importance of multiple regions of the CyRE. These findings show that regions within the CyRE distinct from the **STAT** site are important to the LIF-dependent regulation of VIP gene expression and enable the CyRE to respond in a cell-specific and cytokine-specific manner.

7/3,AB/32 (Item 32 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08597049 95355467 PMID: 7543102

Signal transduction by a CD16/CD7/Jak2 fusion protein.

Sakai I; Nabell L; Kraft A S

Department of Medicine, University of Alabama, Birmingham 35223.

Journal of biological chemistry (UNITED STATES) Aug 4 1995, 270

(31) p18420-7, ISSN 0021-9258 Journal Code: 2985121R

Contract/Grant No.: DK44741; DK; NIDDK; DK48882; DK; NIDDK

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The addition of interleukin-3 (IL-3) and granulocyte-macrophage colony-stimulating **factor** (GM-CSF) to hormone-dependent cells induces tyrosine phosphorylation of Janus protein kinase 2 (Jak2) and activates its in vitro kinase activity. To explore the role of Jak2 in IL-3/GM-CSF-mediated signal transduction, we constructed a CD16/CD7/Jak2 (CD16/Jak2) fusion gene containing the external domain of CD16 and the entire Jak2 molecule and expressed this fusion protein using a recombinant vaccinia virus. The clustering of CD16/Jak2 fusion protein by cross-linking with an anti-CD16 antibody induced autophosphorylation of the fusion protein but did not induce the phosphorylation of either the endogenous Jak2 or the beta chain. Cross-linking of CD16/Jak2 stimulates the tyrosine

phosphorylation of a large group of proteins that are also phosphorylated after the addition of IL-3 or GM-CSF and include proteins of 145, 97, 67, 52, and 42 kDa. Closer analysis demonstrated that the CD16/Jak2 phosphorylates Shc, a 52-kDa protein, and the 145-kDa protein associated tightly with Shc, as well as mitogen-associated protein kinase (pp42). Electrophoretic mobility shift assays demonstrate that CD16/Jak2 activates the ability of signal transduction and activation of **transcription** (**STAT**) proteins to bind to an interferon-gamma-activated sequence **oligonucleotide** in a manner similar to that seen after IL-3 treatment. Cross-linking of the CD16/Jak2 protein stimulated increases in c-fos and junB similar to IL-3 but did not cause major changes in the levels of the c-myc message, which normally increases after IL-3 treatment. Thus, a transmembrane CD16/Jak2 fusion is capable of activating protein phosphorylation and mRNA **transcription** in a manner similar but not identical to hematopoietic growth factors.

7/3,AB/33 (Item 33 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08537452 95295730 PMID: 7776967

In vivo growth hormone treatment rapidly stimulates the tyrosine phosphorylation and activation of Stat3.

Gronowski A M; Zhong Z; Wen Z; Thomas M J; Darnell J E; Rotwein P
Department of Biochemistry and Molecular Biophysics, Washington
University School of Medicine, St. Louis, Missouri 63110, USA.

Molecular endocrinology (Baltimore, Md.) (UNITED STATES) Feb 1995
, 9 (2) p171-7, ISSN 0888-8809 Journal Code: 8801431

Contract/Grant No.: 5-RO1-DK-37449; DK; NIDDK; DK-07120; DK; NIDDK

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The mechanisms by which GH regulates gene expression to alter growth and metabolism are unknown. We have demonstrated previously that in vivo GH treatment rapidly stimulates the tyrosine phosphorylation of multiple nuclear proteins and have identified the inducible **transcription factor** Stat1 (formerly Stat91) as one of the major GH-activated nuclear phosphoproteins. We now show that Stat3, a new member of the **STAT** (signal transducer and activator of **transcription**) family of **transcription** factors, is also phosphorylated on tyrosine residues and rapidly appears in the nucleus in response to GH. Activated Stat3 interacts with the naturally occurring c-sis-inducible element of the c-fos gene after GH treatment, as demonstrated by gel mobility shift assay, and is a component of gel-shifted bands A and B when the high affinity sis-inducible element is used as a probe. Our results suggest that multiple **STAT** proteins may mediate some of the pleiotropic effects of GH on gene expression.

7/3,AB/34 (Item 34 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08535373 95293230 PMID: 7774815

A **STAT** protein domain that determines DNA sequence recognition suggests a novel DNA-binding domain.

Horvath C M; Wen Z; Darnell J E
Laboratory of Molecular Cell Biology, Rockefeller University, New York,
New York 10021, USA.

Genes & development (UNITED STATES) Apr 15 1995, 9 (8) p984-94
, ISSN 0890-9369 Journal Code: 8711660

Contract/Grant No.: AI32489; AI; NIAID; AI34420; AI; NIAID; NS09230; NS;
NINDS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Stat1 and Stat3 are two members of the ligand-activated **transcription factor** family that serve the dual functions of signal transducers and activators of **transcription**. Whereas the two proteins select very similar (not identical) optimum binding sites from random **oligonucleotides**, differences in their binding affinity were readily apparent with natural **STAT**-binding sites. To take advantage of these different affinities, chimeric Stat1:Stat3 molecules were used to locate the amino acids that could discriminate a general binding site from a specific binding site. The amino acids between residues approximately 400 and approximately 500 of these approximately 750-amino-acid-long proteins determine the DNA-binding site specificity. Mutations within this region result in **Stat** proteins that are activated normally by tyrosine phosphorylation and that dimerize but have greatly reduced DNA-binding affinities.

7/3,AB/35 (Item 35 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08425935 95180411 PMID: 7875295

Colony-stimulating factors and interferon-gamma activate a protein related to MGF-Stat 5 to cause formation of the differentiation-induced **factor** in myeloid cells.

Barahmand-pour F; Meinke A; Eilers A; Gouilleux F; Groner B; Decker T
Vienna Biocenter, Institute of Microbiology and Genetics, Austria.

FEBS letters (NETHERLANDS) Feb 20 1995, 360 (1) p29-33, ISSN
0014-5793 Journal Code: 0155157

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The Jak-Stat pathway of intracellular signals is used by growth **factor**- and cytokine receptors to induce gene **transcription**. We have recently reported that differentiation of myeloid cells, induced by phorbol ester, interferon-gamma (IFN-gamma) or colony-stimulating **factor** -1 (CSF-1) is accompanied by the activation of the differentiation-induced **factor** (DIF). Activated DIF specifically associates with a subclass of gamma-interferon activation site (GAS)-like DNA elements. We now report that GM-CSF, which like CSF-1 promotes the generation of mature macrophages, activates DIF. No activation was observed after treatment with the granulocyte growth and differentiation **factor** G-CSF. Antibodies raised against a **Stat** family protein, designated mammary gland **factor**-Stat 5 (MGF-Stat 5), reacted with DIF induced by either CSF-1, GM-CSF or IFN-gamma. Antisera to other known Stats were without effect on the DIF complex in electrophoretic mobility shift assays (EMSA). A 112 kDa protein could be isolated from either GM-CSF- or IFN-gamma-treated cells by GAS **oligonucleotide** precipitation. This protein reacted with antibodies to both MGF-Stat 5 and phosphotyrosine. MGF-Stat 5 and closely related proteins thus define a subfamily of **Stat transcription factors** that are present in a variety of cell types and are required for the onset of immediate gene expression in response to differentiating stimuli.

7/3,AB/36 (Item 36 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08257264 95014451 PMID: 7929335

Growth hormone specifically regulates serine protease inhibitor gene **transcription** via gamma-activated sequence-like DNA elements.

Sliva D; Wood T J; Schindler C; Lobie P E; Norstedt G

Center for Biotechnology, Karolinska Institute, NOVUM, Huddinge, Sweden.
Journal of biological chemistry (UNITED STATES) Oct 21 1994, 269
(42) p26208-14, ISSN 0021-9258 Journal Code: 2985121R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Growth hormone activates gene **transcription** of the serine protease inhibitors (SPI) 2.1 and 2.2 by an unknown mechanism. In order to define the promoter regions responsible for this effect and to characterize the **transcription** factors involved, we have performed gel electrophoresis mobility shift assays on nuclear extracts from cell lines transfected with growth hormone receptor cDNA. We have identified a 9-base pair DNA element, the SPI-GLE 1, which forms a complex with nuclear proteins following activation by growth hormone and which, when placed upstream of a minimal thymidine kinase promoter, drives chloramphenicol acetyltransferase expression in a growth hormone-dependent fashion. This element is similar to those from several genes regulated by other cytokines including interferon. The growth hormone-induced complexes formed were dependent on tyrosine phosphorylation but did not contain the interferon-gamma-activated **transcription factor Stat 91**. Competition studies with **oligonucleotides** similar to the SPI-GLE 1 reveal the sequence of a consensus element that specifically binds growth hormone-regulated nuclear proteins.

7/3,AB/37 (Item 37 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08142451 94277082 PMID: 7516473

Disease-activated **transcription factor**: allergic reactions in human skin cause nuclear translocation of **STAT -91** and induce synthesis of keratin K17.

Jiang C K; Flanagan S; Ohtsuki M; Shuai K; Freedberg I M; Blumenberg M
Ronald O. Perelman Department of Dermatology, New York University Medical Center, New York 10016.

Molecular and cellular biology (UNITED STATES) Jul 1994, 14 (7)
p4759-69, ISSN 0270-7306 Journal Code: 8109087

Contract/Grant No.: AR30682; AR; NIAMS; AR39176; AR; NIAMS; AR39749; AR;
NIAMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Epidermal keratinocytes have important immunologic functions, which is apparent during wound healing, in psoriasis, and in allergic and inflammatory reactions. In these processes, keratinocytes not only produce cytokines and growth factors that attract and affect lymphocytes but also respond to the polypeptide factors produced by the lymphocytes. Gamma interferon (IFN-gamma) is one such signaling polypeptide. Its primary molecular effect is activation of specific **transcription** factors that regulate gene expression in target cells. In this work, we present a molecular mechanism of lymphocyte-keratinocyte signaling in the epidermis. We have induced cutaneous delayed-type hypersensitivity reactions that are associated with an accumulation of lymphocytes. These resulted in activation and nuclear translocation of **STAT -91**, the IFN-gamma-activated **transcription factor**, in keratinocytes in vivo and subsequent induction of **transcription** of keratin K17. Within the promoter of the K17 keratin gene, we have identified and characterized a site that confers the responsiveness to IFN-gamma and that binds the **transcription factor STAT -91**. Other keratin gene promoters tested were not induced by IFN-gamma. These results characterize at the molecular level a signaling pathway produced by the infiltration of lymphocytes in skin and resulting in the specific alteration of gene

expression in keratinocytes.

7/3,AB/38 (Item 38 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08142407 94277038 PMID: 8007943

Stat4, a novel gamma interferon activation site-binding protein expressed in early myeloid differentiation.

Yamamoto K; Quelle F W; Thierfelder W E; Kreider B L; Gilbert D J; Jenkins N A; Copeland N G; Silvennoinen O; Ihle J N

Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, Tennessee 38105.

Molecular and cellular biology (UNITED STATES) Jul 1994, 14 (7)

p4342-9, ISSN 0270-7306 Journal Code: 8109087

Contract/Grant No.: NO1 CO-74101; CO; NCI; P30 CA21765; CA; NCI; RO1 DK42932; DK; NIDDK

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Interferon regulation of gene expression is dependent on the tyrosine phosphorylation and activation of the DNA-binding activity of two related proteins of 91 kDa (STAT1) and/or 113 kDa (STAT2). Recent studies have suggested that these proteins are substrates of Janus kinases and that proteins related in STAT1 are involved in a number of signalling pathways, including those activated in myeloid cells by erythropoietin and interleukin-3 (IL-3). To clone **STAT**-related proteins from myeloid cells, degenerate **oligonucleotides** were used in PCRs to identify novel family members expressed in myeloid cells. This approach allowed the identification and cloning of the Stat4 gene, which is 52% identical to STAT1. Unlike STAT1, Stat4 expression is restricted but includes myeloid cells and spermatogonia. In the erythroid lineage, Stat4 expression is differentially regulated during differentiation. Functionally, Stat4 has the properties of other **STAT** family genes. In particular, cotransfection of expression constructs for Stat4 and Jak1 and Jak2 results in the tyrosine phosphorylation of Stat4 and the acquisition of the ability to bind to the gamma interferon (IFN-gamma)-activated sequence of the interferon regulatory **factor** 1 (IRF-1) gene. Stat4 is located on mouse chromosome 1 and is tightly linked to the Stat1 gene, suggesting that the genes arose by gene duplication. Unlike Stat1, neither IFN-alpha nor IFN-gamma activates Stat4. Nor is Stat4 activated in myeloid cells by a number of cytokines, including erythropoietin, IL-3, granulocyte colony-stimulating **factor**, stem cell **factor**, colon-stimulating **factor** 1, hepatocyte growth **factor**, IL-2, IL-4, and IL-6.

7/3,AB/39 (Item 1 from file: 5)
DIALOG(R) File 5:Biosis Previews(R)
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10533408 BIOSIS NO.: 199699154553

Interleukin-7 signaling in human B cell precursor acute lymphoblastic leukemia cells and murine BAF3 cells involves activation of STAT1 and STAT5 mediated via the interleukin-7 receptor alpha chain.

AUTHOR: Van Der Plas D C; Smiers F; Pouwels K; Hoefsloot L H; Lowenberg B; Touw I P

AUTHOR ADDRESS: Inst. Hematol., Erasmus Univ., P.O. Box 1738, 3000 DR Rotterdam**Netherlands

JOURNAL: Leukemia (Basingstoke) 10 (8):p1317-1325 1996

ISSN: 0887-6924

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Interleukin-7 (IL-7) stimulates the proliferation of normal and leukemic B and T cell precursors and T lymphocytes. Activation of the JAK/STAT pathway has been implicated in IL-7R signaling. We investigated which **STAT** complexes are formed upon stimulation of B cell precursor acute lymphoblastic leukemia (BCP-ALL) cells with IL-7. Gel retardation assays with **STAT-binding oligonucleotides** showed that IL-7 induces the formation of two major **STAT** complexes in BCP-ALL cells. Supershifts with anti-**STAT** antibodies identified these as STAT1 and STAT5 complexes. This pattern of **STAT** activation was seen in all BCP-ALL cases that respond to IL-7 in proliferation assays. IL-7 also induced **STAT/DNA** binding in BCP-ALL cases that failed to proliferate in response to IL-7, suggesting that the ability of IL-7R to activate the JAK/STAT pathway per se is not sufficient for proliferation induction. To determine the contribution of the cytoplasmic domain of the IL-7 receptor α chain (IL-7R- α) to activation of **STAT** proteins, transfectants of the murine pro-B cell line BAF3 were made that express chimeric receptors consisting of the extracellular domain of human granulocyte colony-stimulating factor receptor (G-CSF-R) and the transmembrane and intracellular domains of human IL-7R- α . Activation of the chimeric G-CSF-R/IL-7R- α with G-CSF resulted in a full proliferative response and induced the phosphorylation of JAK1 but not JAK2. Major **STAT** complexes activated by G-CSF-R/IL-7R- α contained STAT1 or STAT5, while some formation of STAT3-containing complexes was also seen. These findings establish that STAT1 and STAT5, and possibly STAT3, are activated upon stimulation of precursor B cells with IL-7. The data further indicate that the IL-7R- α chains are directly involved in the activation of JAKs and STATs and have a major role in proliferative signaling in precursor B cells.

1996

7/3,AB/40 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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10238957 BIOSIS NO.: 199698693875

Prolactin induction of the α -2-macroglobulin gene in rat ovarian granulosa cells: **Stat** 5 activation and binding to the interleukin-6 response element.

AUTHOR: Dajee Maya; Kazansky Alexander V; Raught Brian; Hocke Gertrud M; Fey Georg H; Richards Joanne S(a)

AUTHOR ADDRESS: (a)Dep. Cell Biol., Baylor Coll. Med., One Baylor Plaza, Houston, TX 77030**USA

JOURNAL: Molecular Endocrinology 10 (2):p171-184 1996

ISSN: 0888-8809

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: α -2-Macroglobulin (α -2M) is expressed at high levels in the corpus luteum of pregnant rats in response to PRL and rat placental lactogens. These studies document that PRL induction of α -2M mRNA occurs rapidly in granulosa cells differentiated to the preovulatory phenotype in the presence of FSH and steroid, is hormone specific (induced by PRL but not by LH or interleukin-6 (IL-6)), and involves tyrosine kinase activity. To analyze the cellular signaling events stimulated by PRL, transient transfections of granulosa cells and electrophoretic mobility shift assays were done using the IL-6 response element (IL-6RE) of the α -2M promoter. The IL-6RE consists of two gamma-activating like sequences (GAS) that bind the acute phase response factor (APRF/**Stat** 3) in rat liver and the mammary gland

factor (MGF/**Stat** 5) from mammary tissue. By transfecting various alpha-2M promoter-luciferase reporter transgenes into the granulosa cell cultures, we show that the GAS-like sites together with the minimal -48 base pairs of the alpha-2M promoter can confer PRL inducibility to the luciferase reporter gene. These same GAS-like sequences of the alpha-2M promoter were used to analyze the DNA-binding activity of proteins in whole cell extracts prepared from differentiated granulosa cells exposed to PRL for 0.25, 0.5, 4, and 20 h. PRL rapidly stimulated the binding of a specific protein to labeled alpha-2M GAS-like **oligonucleotide**, and this PRL-induced binding activity was shown to contain **Stat** 5 but not **Stat** 1 or **Stat** 3, using specific antibodies in the electrophoretic mobility shift assays. Because both **Stat** 5 and **Stat** 3 proteins are present in the whole cell extracts of differentiated granulosa cells, PRL appears to activate detectable amounts of **Stat** 5 (and not **Stat** 3). Thus, the initial induction of the alpha-2M gene by PRL in differentiated rat granulosa cells involves, at least in part, the activation (tyrosine phosphorylation?) of **Stat** 5.

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E6	139	AU=ZUCKERMAN K S
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E8	35	AU=ZUCKERMAN KENNETH S
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3/3,AB/1 (Item 1 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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10699356 20235106 PMID: 10775036

Prolonged activation of the mitogen-activated protein kinase pathway is required for macrophage-like differentiation of a human myeloid leukemic cell line.

Hu X; Moscinski L C; Valkov N I; Fisher A B; Hill B J; **Zuckerman K**

S

Department of Internal Medicine, University of South Florida and H. Lee Moffitt Cancer Center and Research Institute, Tampa 33612, USA.

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Cell growth & differentiation : the molecular biology journal of the American Association for Cancer Research (UNITED STATES) Apr 2000, 11 (4) p191-200, ISSN 1044-9523 Journal Code: 9100024

Contract/Grant No.: P30CA76292; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The role of the mitogen-activated protein kinase (MAPK) signal transduction pathway in the proliferation of mammalian cells has been well established. However, there are relatively few reports concerning cell differentiation being mediated by MAPK. The effect of phorbol 12-myristate 13-acetate (PMA) on cell differentiation and signal transduction in a human myeloid leukemia cell line, TF-1a, was investigated. When TF-1a cells were treated with 10^{-6} , 10^{-7} , 10^{-8} , and 10^{-9} M PMA for 24 h, they underwent 98, 93, 91, and 51% macrophage-like differentiation, respectively. PMA treatment rapidly (10 min) induced phosphorylation of MAPK kinase (MEK and p44/42 MAPK), which persisted for at least 24 h. p44/42 MAPK immunoprecipitates from lysates of PMA-treated cells had increased ability to phosphorylate the transcription factor Elk-1. This is important because phosphorylated Elk-1 can be considered an "end-product" of the MAPK pathway. In contrast, treatment of TF-1a cells with granulocyte/macrophage-colony stimulating factor induced only transient activation of MEK and p44/42 MAPK (10-20 min) and an increase (approximately 50%) in cell proliferation, without any change in cellular differentiation. These results suggest that macrophage-like differentiation may be dependent on prolonged activation of the MAPK pathway. Additional support for this conclusion was obtained from experiments showing that treatment of TF-1a cells with antisense oligonucleotides for MEK1 coding sequences prior to adding PMA inhibited macrophage-like differentiation. Furthermore, transient transfection with an inactive, dominant-negative MEK mutant also inhibited PMA-induced differentiation, whereas transient transfection with a plasmid coding for constitutively activated MEK led to macrophage-like differentiation in the absence of PMA.

3/3,AB/2 (Item 2 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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Constitutive activation of the JAK2/STAT5 signal transduction pathway correlates with growth factor independence of megakaryocytic leukemic cell lines.

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The factor-independent Dami/HEL and Meg-01 and factor-dependent Mo7e leukemic cell lines were used as models to investigate JAK/STAT signal transduction pathways in leukemic cell proliferation. Although Dami/HEL and Meg-01 cell proliferation in vitro was independent of and unresponsive to exogenous cytokines including granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-3 (IL-3), IL-6, thrombopoietin (TPO), and tumor necrosis factor-alpha (TNF-alpha), the growth of Mo7e cells was

dependent on hematopoietic growth factors. When these cell lines were cultured in medium without cytokines, a constitutively activated STAT-like DNA-binding factor was detected in nuclear extracts from both Dami/HEL and Meg-01 cells. However, the STAT-like factor was not detectable in untreated Mo7e cells, but was activated transiently in Mo7e cells in response to cytokine treatments. The constitutively activated and cytokine-induced STAT-like DNA-binding factor in these three cell lines was identified as STAT5 by oligonucleotide competition gel mobility assays and by specific anti-STAT antibody gel supershift assays. Constitutive activation of JAK2 also was detected in the factor-independent cell lines, but not in Mo7e cells without cytokine exposure. Meg-01 cells express a p185 BCR/ABL oncogene, which may be responsible for the constitutive activation of STAT5. Dami/HEL cells do not express the BCR/ABL oncogene, but increased constitutive phosphorylation of Raf-1 oncoprotein was detected. In cytokine bioassays using growth factor-dependent Mo7e and TF-1 cells as targets, conditioned media from Dami/HEL and Meg-01 cells did not show stimulatory effects on cell proliferation. Our results indicate that the constitutive activation of JAK2/STAT5 correlates with the factor-independent growth of Dami/HEL and Meg-01 cells. The constitutive activation of JAK2/STAT5 in Dami/HEL cells is triggered by a mechanism other than autocrine cytokines or the BCR/ABL oncoprotein.

3/3,AB/3 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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13092143 BIOSIS NO.: 200100299292

Antisense **oligonucleotides** targeting human STAT5 inhibit the proliferation of human leukemia cell lines that express constitutively activated STAT5.

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RECORD TYPE: Abstract

LANGUAGE: English

SUMMARY LANGUAGE: English

ABSTRACT: We reported previously that constitutively activated STAT5 was correlated with the growth factor-independence of HEL, Meg-01 and K562 leukemia cell lines (Blood 93:2369,1999). Data from our lab and others have shown that constitutively activated STAT5 was detected in about 50% of primary acute myeloid leukemia patients. We and others further demonstrated the critical role of the constitutively activated STAT5 in the survival of these cell lines, by overexpression of dominant-negative STAT5. All of these data indicate that STAT5 represents an attractive but technically challenging drug discovery target. Although activation of the JAK/STAT signaling pathway can be inhibited by a small chemical AG490, this tyrphostin molecule has an extremely short in vivo half-life and, at higher doses, has non-specific effects on cell proliferation. In the present report, we describe the identification of antisense **oligonucleotides** to STAT5 and their effects on proliferation of HEL and Meg-01 cells. A limited gene-walk was performed with phosphorothioate deoxynucleotides to identify sequences capable of downregulating STAT and downstream gene expression. The **oligonucleotides** were designed according to the following principles: (1) 18-base antisense sequence, (2) complementary to at least five bases that are not hybridized in the

predicted secondary mRNA structure, (3) minimal cytosine-guanine motifs, and (4) less than four contiguous thymine or guanine bases. A chimeric antisense **oligonucleotide** (S-AS-T53), which has complementary sequence for both STAT5A and STAT5B, demonstrated the highest level of inhibitory activity. Incubation of HEL cells (human erythroleukemia cell line) with S-AS-T53 caused a dose-dependent STAT5A and STAT5B protein downregulation (50% inhibition = 1.2 μ M S-AS-T53), but had no effect on closely related members of the STAT family, STAT3 and STAT1. The treatment also inhibited the expression of the downstream STAT5-regulated Bcl-XL gene. Furthermore, treatment of HEL and Meg-01 cells with S-AS-T53 inhibited cell proliferation in a dose-dependent manner (50% inhibition = 0.86 and 0.63 μ M S-AS-T53, respectively). These data further demonstrate the critical role of the constitutively activated STAT5 in HEL and Meg-01 leukemic cell growth. S-AS-T53 may provide an alternative approach to small chemical compounds for inhibiting survival and proliferative signaling in leukemic cells with constitutively activated STAT5.

2000

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DIALOG(R)File 5:Biosis Previews(R)
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11104228 BIOSIS NO.: 199799725373

A STAT5-binding, double-stranded **oligonucleotide** inhibits the proliferation of factor-independent leukemia cell lines, DAMI/HEL and MEG-01.

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